APPENDIX A

PBPK Modeling of TCE and Metabolites—Detailed Methods and Results

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APPENDIX A. PBPK MODELING OF TCE AND METABOLITES–DETAILED METHODS AND RESULTS

A.1. THE HIERARCHICAL BAYESIAN APPROACH TO CHARACTERIZING PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL UNCERTAINTY AND VARIABILITY

The Bayesian approach for characterizing uncertainty and variability in PBPK model parameters, used previously for trichloroethylene (TCE) in Bois (2000a, b) and Hack et al. (2006), is briefly described here as background. Once a physiologically based pharmacokinetic (PBPK) model structure is specified, characterizing the model reduces to calibrating and making inferences about model parameters. The use of least-squares point estimators is limited by the large number of parameters and small amounts of data. The use of least-squares estimation is reported after imposing constraints for several parameters (Fisher, 2000; Clewell et al., 2000). This is reasonable for a first estimate, but it is important to follow-up with a more refined treatment. This is implemented by a Bayesian approach to estimate posterior distributions on the unknown parameters, a natural choice, and almost a compulsory consequence given the large number of parameters and relatively small amount of data, and given the difficulties of frequentist estimation in this setting.

As described by Gelman et al. (1996), the Bayesian approach to population PBPK modeling involves setting up the overall model in several stages. A nonlinear PBPK model, with predictions denoted f, describes the absorption, distribution, metabolism, and excretion of a compound and its metabolites in the body. This model depends on several, usually known, parameters such as measurement times t, exposure E, and measured covariates φ. Additionally, each subject i in a population has a set of unmeasured parameters θ_i. A random effects model describes their population variability $P(\theta_i \mid \mu, \Sigma^2)$, and a prior distribution $P(\mu, \Sigma^2)$ on the population mean μ and covariance Σ^2 (often assumed to be diagonal) incorporates existing scientific knowledge about them. Finally, a "measurement error" model $P(y \mid f[\theta, \varphi, E, t], \sigma^2)$ describes deviations (with variance σ^2) between the data y and model predictions f (which of course depends on the unmeasured parameters θ_i and the measured parameters t, E, and φ). This "measurement error" level of the hierarchical model typically also encompasses intraindividual variability as well as model misspecification, but for notational convenience we refer to it here as "measurement error." Because these other sources of variance are lumped into a single "measurement error," a prior distribution of its variance σ^2 must be specified even if the actual analytic measurement error is known. All these components are illustrated graphically in Figure A-1.

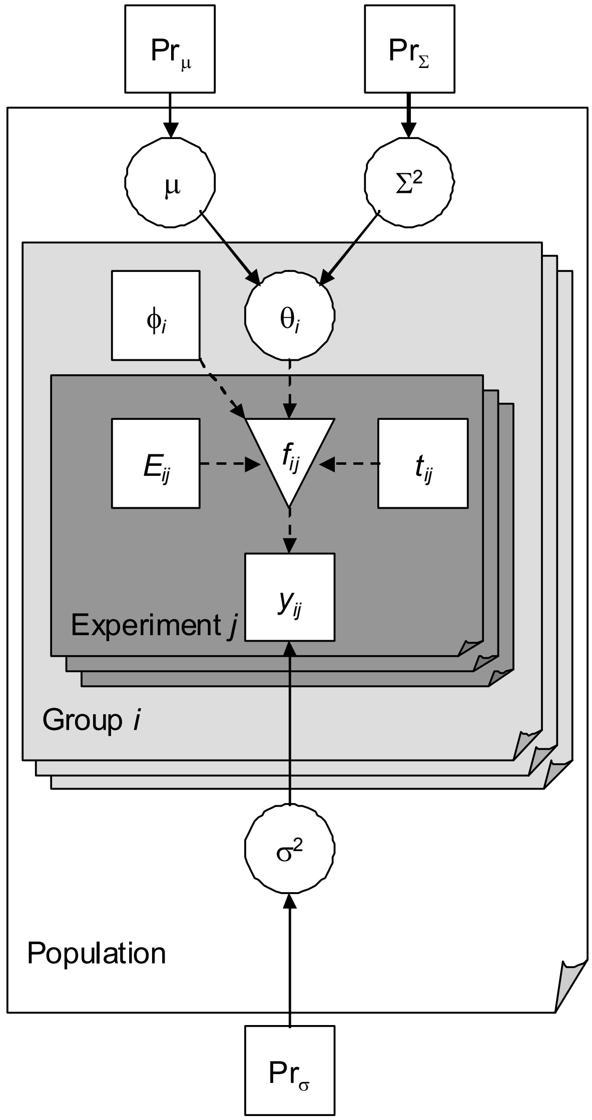

Figure A-1. Hierarchical population statistical model for PBPK model parameter uncertainty and variability (see Gelman et al., 1996). Square nodes denote fixed or observed quantities; circle notes represent uncertain or unobserved quantities, and the nonlinear model outputs are denoted by the inverted triangle. Solid arrows denote a stochastic relationship represented by a conditional distribution [$A \rightarrow B$ means $B \sim P(B|A)$], while dashed arrows represent a function relationship [$B = f(A)$]. The population consists of groups (or subjects) i, each of which undergoes one or more experiments j with exposure parameters E_{ij} with data y_{ij} collected at times t_{ij}. The PBPK model produces outputs f_{ij} for comparison with the data y_{ij}. The difference between them ("measurement error") has variance σ^2, with a fixed prior distribution Pr, which in this case is the same for the entire population. The PBPK model also depends on measured covariates ϕ_i (e.g., body weight) and unobserved model parameters θ_i (e.g., V_{MAX}). The parameters θ_i are drawn from a population with mean μ and variance Σ^2, each of which is uncertain and has a prior distribution assigned to it.

The posterior distribution for the unknown parameters is obtained in the usual manner by multiplying (1) the prior distribution for the population mean and variance and the "measurement" error $P(\mu, \Sigma^2)\ P(\sigma^2)$, (2) the population distribution for the individual parameters $P(\theta \mid \mu, \Sigma^2)$, and (3) the likelihood $P(y \mid \theta, \sigma^2)$, where for notational convenience, the dependence on f, φ, E, and t (which are taken as fixed for a given dataset) is dropped:

$$P(\theta, \mu, \Sigma^2, \sigma^2 \mid y) \propto P(\mu, \Sigma^2)\ P(\sigma^2)\ P(\theta \mid \mu, \Sigma^2)\ P(y \mid \theta, \sigma^2) \qquad \text{(Eq. A-1)}$$

Here, each subject's parameters θ_i have the same sampling distribution (i.e., they are independently and identically distributed), so their joint prior distribution is

$$P(\theta \mid \mu, \Sigma^2) = \prod\nolimits_{i=1\ldots n} P(\theta_i \mid \mu, \Sigma^2) \qquad \text{(Eq. A-2)}$$

Different experiments $j = 1\ldots n_j$ may have different exposure and different data collected and different time points. In addition, different types of measurements $k = 1\ldots n_k$ (e.g., TCE blood, TCE breath, trichloroacetic acid [TCA] blood, etc.) may have different errors, but errors are otherwise assumed to be iid. Since the individuals are treated as independent given $\theta_{1\ldots n}$, the total likelihood function is simply

$$P(y \mid \theta, \sigma^2) = \prod\nolimits_{i=1\ldots n} \prod\nolimits_{j=1\ldots nij} \prod\nolimits_{k=1\ldots m} \prod\nolimits_{l=1\ldots Nijk} P(y_{ijkl} \mid \theta_i, \sigma_k^2, t_{ijkl}) \qquad \text{(Eq. A-3)}$$

where n is the number of subjects, n_{ij} is the number of experiments in that subject, m is the number of different types of measurements, N_{ijk} is the number (possibly 0) of measurements (e.g., time points) for subject i of type k in experiment j, and t_{ijkl} are the times at which measurements for individual i of type k were made in experiment j.

Given the large number of parameters, complex likelihood functions, and nonlinear PBPK model, Markov chain Monte Carlo (MCMC) simulation was used to generate samples from the posterior distribution. An important practical advantage of MCMC sampling is the ability to implement inference in nearly any probability model and the possibility to report inference on any event of interest. MCMC simulation was introduced by Gelfand and Smith (1990) as a generic tool for posterior inference. See Gilks et al. (1996) for a review. In addition, because many parameters are allowed to vary simultaneously, the local parameter sensitivity analyses often performed with PBPK models (in which the changes in model predictions are assessed with each parameter varied by a small amount) are unnecessary.[1] In the context of PBPK models, the MCMC simulation can be carried out as described by Hack et al. (2006). The simulation program MCSim (version 5.0.0) was used to implement MCMC posterior simulation, with analysis of the results performed using the *R* statistical package. Simulation-based parameter estimation with MCMC posterior simulation gives rise to an additional source of uncertainty. For instance, averages computed from the MCMC simulation output represent the desired posterior means only asymptotically, in the limit as the number of iterations goes to infinity. Any implementation needs to include a convergence diagnostic to judge practical convergence. The potential scale-reduction-factor convergence diagnostic *R* of Gelman et al. (1996) was used here, as it was in Hack et al. (2006).

A.2. EVALUATION OF THE HACK ET AL. (2006) PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL

U.S. Environmental Protection Agency (U.S. EPA) obtained the original model code for the version of the TCE PBPK model published in Hack et al. (2006) and conducted a detailed evaluation of the model, focusing on the following areas: convergence, posterior estimates for model parameters, and comparison of model predictions with *in vivo* data.

A.2.1. Convergence

As noted in Hack et al. (2006), the diagnostics for the MCMC simulations (3 chains of length 20,000−25,000 for each species) indicated that additional samples might further improve convergence. A recent analysis of tetrachloroethylene pharmacokinetics indicated the need to be especially careful in ensuring convergence (Chiu and Bois, 2006). Therefore, the number of MCMC samples per chain was increased to 75,000 for rats (first 25,000 discarded) and 175,000 for mice and humans (first 75,000 discarded). Using these chain lengths, the vast majority of the parameters had potential scale reduction factors $R \leq 1.01$, and all population parameters had $R \leq 1.05$, indicating that longer chains would be expected to reduce the standard deviation (or other measure of scale, such as a confidence interval) of the posterior distribution by less than this factor (Gelman et al., 2004).

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[1] In particular, local sensitivity analyses are typically used to assess the impact of alternative parameter estimates on model predictions, inform experimental design, or assist prioritizing risk assessment research. Only the first purpose is relevant here; however, the full uncertainty and variability analysis allows for a more comprehensive assessment than can be done with sensitivity analyses. Separately, such analyses could be done to design experiments and prioritize research that would be most likely to help reduce the remaining uncertainties in TCE toxicokinetics, but that is beyond the scope of this assessment.

In addition, analysis of autocorrelation within chains using the R-CODA package (Plumber et al., 2008) indicated that there was significant serial correlation, so additional "thinning" of the chains was performed in order to reduce serial correlations. In particular, for rats, for each of three chains, every 100th sample from the last 50,000 samples was used; and for mice and humans, for each of three chains, every 200th sample from the last 100,000 samples was used. This thinning resulted in a total of 1,500 samples for each species available for use for posterior inference.

Finally, an evaluation was made of the "convergence" of dose metric predictions—that is, the extent to which the standard deviation or confidence intervals for these predictions would be reduced with additional samples. This is analogous to a "sensitivity analysis" performed so that most effort is spent on parameters that are most influential in the result. In this case, the purpose is to evaluate whether one can sample chains only long enough to ensure convergence of predictions of interest, even if certain more poorly identified parameters take longer chains to converge. The motivation for this analysis is that for a more complex model, running chains until all parameters have $R \leq 1.01$ or 1.05 may be infeasible given the available time and resource. In addition, as some of the model parameters had prior distributions derived from "visual fitting" to the same data, replacing those distributions with less informative distributions (in order to reduce bias from "using the same data twice") may require even longer chains for convergence.

Indeed, it was found that R-values for dose metric predictions approached one more quickly than PBPK model input parameters. The most informative simulations were for mice, which converged the slowest and, thus, had the most potential for convergence-related error. Results for rats could not be assessed because the model converged so rapidly, and results for humans were similar to those in mice, though the deviations were all less because of the more rapid convergence. In the mouse model, after 25,000 iterations, many PBPK model parameters had R-values >2, with more than 25% greater than 1.2. However, all dose metric predictions had $R < 1.4$, with the more than 96% of then <1.2 and the majority of them <1.01. In addition, when compared to the results of the last 100,000 iterations (after the total of 175,000 iterations), more than 90% of the medians estimates shifted by less than 20%, with the largest shifts less than 40% (for glutathione [GSH] metabolism dose metrics, which had no relevant calibration data). Tail quantiles had somewhat larger shifts, which was expected given the limited number of samples in the tail, but still more than 90% of the 2.5 and 97.5 percentile quantiles had shifts of less than 40%. Again, the largest shifts, on order of 2-fold, were for GSH-related dose metrics that had high uncertainty, so the relative impact of limited sample size is small.

Therefore, the additional simulations performed in this evaluation, with 3- to 7-fold longer chains, did not result in much change in risk assessment predictions from the original Hack et al. (2006) results. Thus, assessing prediction convergence appears sufficient for assessing convergence of the TCE PBPK model for the purposes of risk assessment prediction.

A.2.2. Evaluation of Posterior Distributions for Population Parameters

Posterior distributions for the population parameters were first checked for whether they appeared reasonable given the prior distributions. Inconsistency between the prior and posterior distributions may indicate an insufficiently broad prior distribution (i.e., overconfidence in their specification), a mis-specification of the model structure, or an error in the data. Parameters that were flagged for further investigation were those for which the interquartile ranges (intervals bounded by the 25^{th} and 75^{th} percentiles) of the prior and posterior distributions did not overlap. In addition, lumped metabolism and clearance parameters for TCA, trichloroethanol (TCOH), and trichloroethanol-glucuronide conjugate (TCOG) were checked to make sure that they remained physiological—e.g., metabolic clearance was not more than hepatic blood flow and urinary clearance not more than kidney blood flow (constraints that were not present in the Hack et al., 2006 priors).

In mice, population mean parameters that had lack of overlap between priors and posteriors included the affinity of oxidative metabolism ($\ln K_M$), the TCA plasma-blood concentration ratio (lnTCAPlas), the TCE stomach to duodenum transfer coefficient (lnKTSD), and the urinary excretion rates of TCA and TCOG (lnkUrnTCAC and lnkUrnTCOGC). For K_M, this is not unexpected, as previous investigators have noted inconsistency in the K_M values between *in vitro* values (upon which the prior distribution was based) and *in vivo* values derived from oral and inhalation exposures in mice (Abbas and Fisher, 1997; Greenberg et al., 1999). For the other mean parameters, the central estimates were based on visual fits, without any other *a priori* data, so it is reasonable to assume that the inconsistency is due to insufficiently broad prior distributions. In addition, the population variance for the TCE absorption coefficient from the duodenum (kAD) was rather large compared to the prior distribution, likely due to the fact that oral studies included TCE in both oil and aqueous solutions, which are known to have very different absorption properties. Thus, the larger population variance was required to accommodate both of them. Finally, the estimated clearance rate for glucurondiation of TCOH was substantially greater than hepatic blood flow. This is an artifact of the one-compartment model used for TCOH and TCOG, and suggests that first pass effects are important for TCOH glucurondiation. Therefore, the model would benefit from the additional of a separate liver compartment so that first pass effects can be accounted for, particularly when comparing across dose-routes.

In rats, the only population mean or variance parameter for which the posterior distribution was somewhat inconsistent with the prior distribution was the population mean for the $\ln K_M$. While the interquartile regions did not overlap, the 95 percentile regions did, so the discordance was relatively minor. However, as with mice, the estimated clearance rate for glucurondiation of TCOH was substantially greater than hepatic blood flow.

In humans, some of the chemical-specific parameters for which priors were established using visual fits had posterior distributions that were somewhat inconsistent, including the oxidative split between TCA and TCOH, biliary excretion of TCOG (lnkBileC), and the TCOH distribution volume (VBodC). More concerning was the fact that the posterior distributions for several physiological volumes and flows were rather strongly discordant with the priors and/or near their truncation limits, including gut, liver, and slowly perfused blood flow, the volumes of the liver and rapidly perfused compartments. In addition, a number of tissue partition coefficients were somewhat inconsistent with their priors, including those for TCE in the gut, rapidly perfused, and slowly perfused tissues, and TCA in the body and liver. Finally, a number of population variances (for TCOH clearance [ClTCOHC], urinary excretion of TCOG [kUrnTCOGC], ventilation-perfusion ratio [VPR], cardiac output [QCC], fat blood flow and volume [QFatC and VFatC], and TCE blood-air partition coefficient [PB])were somewhat high compared to their prior distributions, indicating much greater population variability than expected.

A.2.3. Comparison of Model Predictions With Data

A schematic of the comparisons between model predictions and data are shown in Figure A-2. In the hierarchical population model, group-specific parameters were estimated for each dataset used in calibrating the model (posterior group-specific θ_i in Figure A-2). Because these parameters are in a sense "optimized" to the experimental data themselves, the group-specific predictions (posterior group-specific y_{ij} in Figure A-2) using these parameters should be accurate by design. Poor fits to the data using these group-parameters may indicate a misspecification of the model structure, prior parameter distributions, or an error in the data. In addition, it is useful to generate "population-based" parameters (posterior population θ) using only the posterior distributions for the population means (μ) and variances (Σ^2), instead of the estimated group-specific parameters. These population predictions provide a sense as to whether the model and the predicted degree of population uncertainty and variability adequately account for the range of heterogeneity in the experimental data. Furthermore, assuming the group-specific predictions are accurate, the population-based predictions are useful to identify whether one or more if the datasets are "outliers" with respect to the predicted population. In addition, a substantial number of *in vivo* datasets was available in all three species that were not previously used for calibration. Thus, it is informative to compare the population-based model predictions, discussed above, to these additional "validation" data in order to assess the predictive power of the PBPK model.

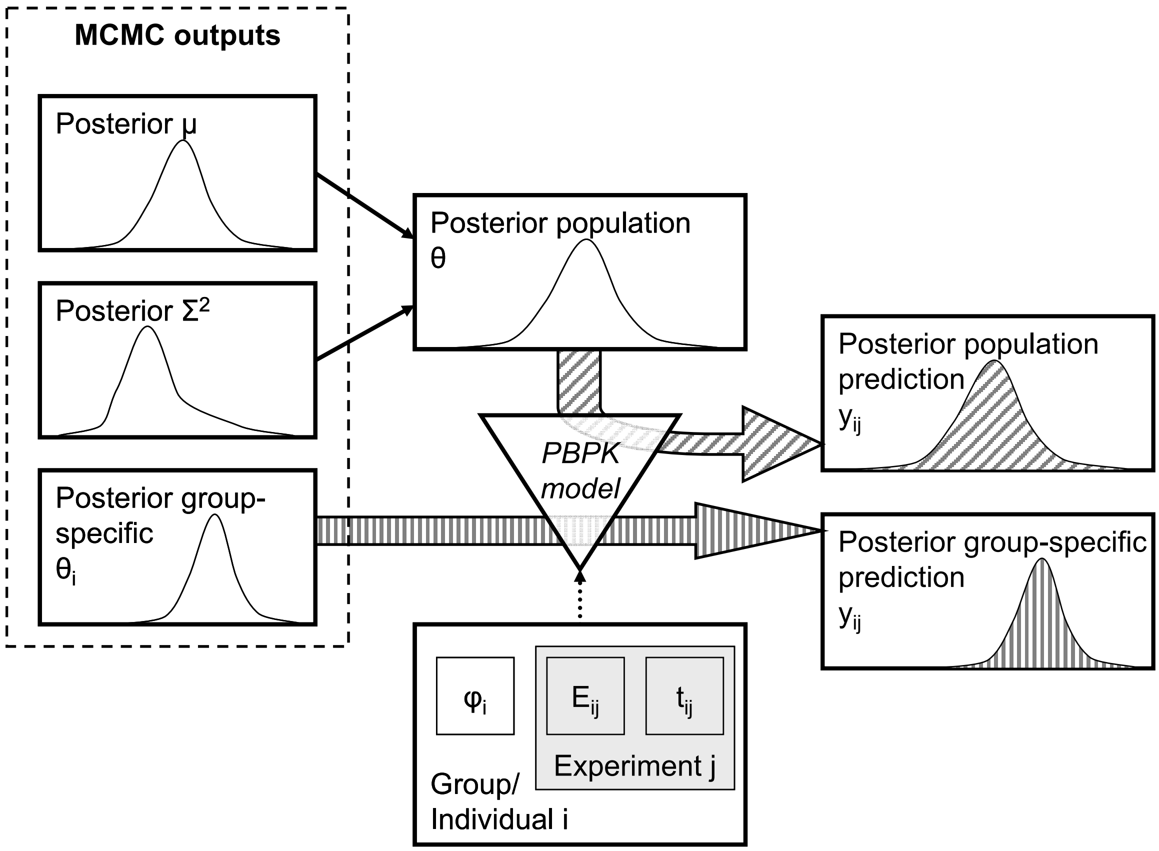

Figure A-2. Schematic of how posterior predictions were generated for comparison with experimental data. Two sets of posterior predictions were generated: population predictions (diagonal hashing) and group-specific predictions (vertical hashing).

A.2.3.1. *Mouse Model*

A.2.3.1.1. ***Group-specific and population-based predictions.*** Initially, the sampled group-specific parameters were used to generate predictions for comparison to the calibration data. Because these parameters were "optimized" for each group, these "group-specific" predictions should be accurate by design. However, unlike for the rat (see below), this was not the case for some experiments (this is partially responsible for the slower convergence). In particular, the predictions for TCE and TCOH concentrations for the Abbas and Fisher (1997) data were poor. In addition, TCE blood concentrations for the Greenberg et al. (1999) data were consistently overpredicted. These data are discussed further in Table A-1.

Next, only samples of the population parameters (means and variances) were used, and "new groups" were sampled from appropriate distributions using these population means and variances. These "new groups" then represent the predicted population distribution, incorporating both variability in the population as well as uncertainty in the population means and variances. These "population-based" predictions were then compared to both the data used in calibration, as well as the additional data identified that was not used in calibration. The PBPK model was modified to accommodate some of the different outputs (e.g., tissue concentrations) and exposure routes (TCE, TCA, and TCOH intravenous [i.v.]) used in the "noncalibration" data, but otherwise it is unchanged.

A.2.3.1.1.1. *Group-specific predictions and calibration data*. [See Appendix.linked.files\AppA.2.3.1.1.1.Hack.mouse.group.calib.TCE.DRAFT.pdf.]

A.2.3.1.1.2. *Population-based predictions and calibration and additional evaluation data*. [See Appendix.linked.files\AppA.2.3.1.1.2.Hack.mouse.pop.calib.eval.TCE.DRAFT.pdf.]

A.2.3.1.2. *Conclusions regarding mouse model.*

A.2.3.1.2.1. *Trichloroethylene (TCE) concentrations in blood and tissues not well-predicted*. The PBPK model for the parent compound does not appear to be robust. Even group-specific fits to datasets used for calibration were not always accurate. For oral dosing data, there is clearly high variability in oral uptake parameters, and the addition of uptake through the first (stomach) compartment should improve the fit. Unfortunately, inaccurate TCE uptake parameters may lead to inaccurately estimated kinetic parameters for metabolites TCA and TCOH, even if current fits are adequate.

Table A-1. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in mice

Reference	Simulation #	Calibration data	Discussion
Abbas et al., 1997	41−42		These data are only published as an abstract. They consist of TCA and TCOH blood and urine data from TCA and TCOH i.v. dosing. Blood levels of TCA and TCOH are fairly accurately predicted. From TCOH dosing, urinary TCOG excretion is substantially overpredicted, and from TCA dosing, urinary TCA excretion is substantially overpredicted.
Abbas and Fisher, 1997	3−6	√	Results for these data were mixed. TCA levels were the best fit. The calibration data included TCA blood and liver data, which were well predicted except at the earliest time-point. In addition, TCA concentrations in the kidney were fairly consistent with the surrogate TCA body concentrations predicted by the model. Urinary TCA was well predicted at the lower two and highest doses, but somewhat underpredicted (though still in the 95% confidence region) at 1,200 mg/kg. TCE levels were in general not well fit. Calibration data included blood, fat, and liver concentrations, which were predicted poorly particularly at early and late times. One reason for this is probably the representation of oral uptake. Although both the current model and the original Abbas and Fisher (1997) model had two-compartments representing oral absorption, in the current model uptake can only occur from the second compartment. By contrast, the Abbas and Fisher (1997) model had uptake from both compartments, with the majority occurring from the first compartment. Thus, the explanation for the poor fit, particularly of blood and liver concentrations, at early times is probably simply due to differences in modeling oral uptake. This is also supported by the fact that the oral uptake parameters tended to be among those that took the longest to converge. Group-specific blood TCOH predictions were poor, with under-prediction at early times and overprediction at late times. Population-based blood TCOH predictions tended to be underpredicted, though generally within the 95% confidence region. Group-specific urinary TCOG predictions were fairly accurate except at the highest dose. These predictions are also probably affected by the apparent misrepresentation of oral uptake. In addition, a problem as found in the calibration data in that data on free TCOH was calibrated against predictions of total TCOH (TCOH+TCOG). A number of TCOH and TCOG measurements were not included in the calibration—among them tissue concentrations of TCOH and tissue and blood concentrations of TCOG. Blood concentrations (the only available surrogate) were poor predictors of tissue concentrations of TCOH and TCOG (model generally under-predicted). For TCOG, this may be due in part to the model assumption that the distribution volume of TCOG is equal to that of TCOH.

Table A-1. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in mice (continued)

Reference	Simulation #	Calibration data	Discussion
Fisher et al., 1991	1−2 (open chamber)	√	Venous blood TCE concentrations were somewhat underpredicted (a common issue with inhalation exposures in mice—see discussion of Greenberg et al., 1999 below), but within the 95% confidence region of both group-specific and population-based predictions. Plasma TCA levels were well predicted, with most of the data near the interquartile region of both group-specific and population-based predictions (but with substantial scatter in the male mice). However, it should be noted that only a single exposure concentration for each sex was used in calibration, with 6 additional exposures (3 for each sex) not included (see simulations 21−26, below).
	7−16 (closed chamber)	√	Good posterior fits were obtained for these data—closed chamber data with initial concentrations from 300 to 10,000 ppm. Some variability in V_{MAX}, however, was noted in the posterior distributions for that parameter. Using group-specific V_{MAX} values resulted in better fits to these data. However, there appears to be a systematic trend of lower estimated apparent V_{MAX} at higher exposures. Similarly, posterior estimates of cardiac output and the ventilation-perfusion ratio declined (slightly) with higher exposures. These could be related to documented physiological changes (e.g., reduced ventilation rate and body temperature) in mice when exposed to some volatile organics.
	21−26 (open chamber, additional exposures)		Data from three additional exposures for each sex were available for comparison to model predictions. Plasma TCA levels were generally well predicted, though the predictions for female mice data showed some systematic over-prediction, particularly at late times (i.e., data showed shorter apparent half-life). Blood TCE concentrations were consistently overpredicted, sometimes by almost an order of magnitude, except in the case of female mice at 236 ppm, for which predictions were fairly accurate.
Fisher and Allen, 1993	31−36		Predictions for these gavage data were generally fairly accurate. There was a slight tendency to overpredict TCA plasma concentrations, with predictions tending to be worse in the female mice. Blood levels of TCE were adequately predicted, though there was some systematic underprediction at 2−6 h after dosing.
Green and Prout, 1985	40		This datum consists of a single measurement of urinary excretion of TCA at 24 h as a fraction of dose, from TCA i.v. dosing. The model substantially over-predicts the amount excreted. Whereas Green and Prout (1985) measured 35% excreted at 24 h, the model predicts virtually complete excretion at 24 h.

Table A-1. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in mice (continued)

Reference	Simulation #	Calibration data	Discussion
Greenberg et al., 1999	17−18	√	The calibration data included blood TCE, TCOH, and TCA data. Fits to blood TCA and TCOH were adequate, but as with the Fisher et al. (1991) inhalation data, TCE levels were overpredicted (outside the 95% confidence region during and shortly after exposure). As with Abbas and Fisher (1997), there were additional data in the study that was not used in calibration, including blood levels of TCOG and tissue levels of TCE, TCA, TCOH, and TCOG. Tissue levels of TCE were somewhat overpredicted, but generally within the 95% confidence region. TCA levels were adequately predicted, and mostly in or near the interquartile region. TCOH levels were somewhat underpredicted, though within the 95% confidence region. TCOG levels, for which blood served as a surrogate for all tissues, were well predicted in blood and the lung, generally within the interquartile region. However, blood TCOG predictions underpredicted liver and kidney concentrations.
Larson and Bull, 1992b	37−39		Blood TCA predictions were fairly accurate for these data. However, TCE and TCOH blood concentrations were underpredicted by up to an order of magnitude (outside the 95% confidence region). Part of this may be due to uncertain oral dosing parameters. Urinary TCA and TCOG were also generally underpredicted, in some cases outside of the 95% confidence region.
Prout et al., 1985	19	√	Fits to these data were generally adequate—within or near the interquartile region.
	27−30 (urinary excretion at different doses)		These data consisted of mass balance studies of the amount excreted in urine and exhaled unchanged at doses from 10 to 2,000 mg/kg. TCA excretion was consistently overpredicted, except at the highest dose. TCOG excretion was generally well predicted—within the interquartile range. The amount exhaled was somewhat overpredicted, with a 4-fold difference (but still within 95% confidence) at the highest dose.
Templin et al., 1993	20	√	Blood TCA levels from these data were well predicted by the model. Blood TCE and TCOH levels were well predicted using group-specific parameters, but did not appear representative using population-derived parameters. However, this is probably a result of the group-specific oral absorption parameter, which was substantially different than the population mean.

The TCE data from inhalation experiments also are not well estimated, particularly blood levels of TCE. While fractional uptake has been hypothesized, direct evidence for this is lacking. In addition, physiologic responses to TCE vapors (reduced ventilation rates, lowered body temperature) are a possibility. These are weakly supported by the closed chamber data, but the amount of the changes is not sufficient to account for the low blood levels of TCE observed in the open chamber experiments. It is also not clear what role presystemic elimination due to local metabolism in the lung may play. It is known that the mouse lung has a high capacity to metabolize TCE (Green et al., 1997). However, in the Hack et al. (2006) model, lung metabolism is limited by flow to the tracheobronchial region. An alternative formulation for lung metabolism in which TCE is available for metabolism directly from inhaled air (similar to that used for styrene, Sarangapani et al., 2003), may allow for greater presystemic elimination of TCE, as well as for evaluating the possibility of wash-in/wash-out effects. Furthermore, the potential impact of other extrahepatic metabolism has not been evaluated. Curiously, predictions for the tissue concentrations of TCE observed by Greenberg et al. (1999) were not as discrepant as those for blood. A number of these hypotheses could be tested; however, the existing data may not be sufficient to distinguish them. The Merdink et al. (1998) study, in which TCE was given by i.v. (thereby avoiding both first pass in the liver and any fractional uptake issue in the lung), may be somewhat helpful, but unfortunately only oxidative metabolite concentrations were reported, not TCE concentrations.

A.2.3.1.2.2. ***Trichloroacetic acid (TCA) blood concentrations well predicted following trichloroethylene (TCE) exposures, but TCA flux and disposition may not be accurate*.** TCA blood and plasma concentrations following TCE exposure are consistently well predicted. However, the total flux of TCA may not be correct, as evidenced by the varying degrees of consistency with urinary excretion data. Of particular importance are TCA dosing studies, none of which were included in the calibration. In these studies, total recovery of urinary TCA was found to be substantially less than the administered dose. However, the current model assumes that urinary excretion is the only source of clearance of TCA, leading to overestimation of urinary excretion. This fact, combined with the observation that under TCE dosing, the model appears to give accurate predictions of TCA urinary excretion for several datasets, strongly suggests a discrepancy in the amount of TCA formed from TCE. That is, since the model appears to overpredict the fraction of TCA that appears in urine, it may be reducing TCA production to compensate. Inclusion of the TCA dosing studies (including some oral dosing studies), along with inclusion of a nonrenal clearance pathway, would probably be helpful in reducing these discrepancies. Finally, improvements in the TCOH/TCOG submodel, below, should also help to ensure accurate estimates of TCA kinetics.

A.2.3.1.2.3. *<u>Trichloroethanol−trichloroethanol-glucuronide conjugate (TCOH/TCOG) submodel requires revision and recalibration</u>*. Blood levels of TCOH and TCOG were inconsistently predicted. Part of this is due to the problems with oral uptake, as discussed above. In addition, the problems identified with the use of the Abbas and Fisher (1997) data (i.e., free TCOH vs. total TCOH), mean that this submodel is not likely to be robust.

An additional concern is the over-prediction of urinary TCOG from the Abbas et al. (1997) TCOH i.v. data. Like the case of TCA, this indicates that some other source of TCOH clearance (not to TCA or urine—e.g., to dichloroacetic acid [DCA] or some other untracked metabolite) is possible. This pathway can be considered for inclusion, and limits can be placed on it using the available data.

Also, like for TCA, the fact that blood and urine are relatively well predicted from TCE dosing strongly suggests a discrepancy in the amount of TCOH formed from TCE. That is, since the model appears to overpredict the fraction of TCOH that appears in urine, it may be reducing TCOH production to compensate. Including the TCOH dosing data would likely be helpful in reducing these discrepancies.

Finally, as with the rat, the model needs to ensure that any first pass effect is accounted for appropriately. Importantly, the estimated clearance rate for glucuronidation of TCOH is substantially greater than hepatic blood flow. As was shown in Okino et al. (2005), in such a situation, the use of a single compartment model across dose routes will be misleading because it implies a substantial first-pass effect in the liver that cannot be modeled in a single compartment model. That is, since TCOH is formed in the liver from TCE, and TCOH is also glucuronidated in the liver to TCOG, a substantial portion of the TCOH may be glucuronidated before reaching systemic circulation. This suggests that a liver compartment for TCOH is necessary. Furthermore, because substantial TCOG can be excreted in bile from the liver prior to systemic circulation, a liver compartment for TCOG may also be necessary to address that first pass effect.

The addition of the liver compartment will necessitate several changes to model parameters. The distribution volume for TCOH will be replaced by two parameters: the liver:blood and body:blood partition coefficients. Similarly for TCOG, liver:blood and body:blood partition coefficients will need to be added. Clearance of TCOH to TCA and TCOG can be redefined as occurring in the liver, and urinary clearance can be redefined as coming from the rest of the body. Fortunately, there are substantial data on circulating TCOG that has not been included in the calibration. These data should be extremely informative in better estimating the TCOH/TCOG submodel parameters.

A.2.3.1.2.4. ***Uncertainty in estimates of total metabolism*.** Closed chamber data are generally thought to provide a good indicator of total metabolism. Both group-specific and population-based predictions of the only available closed chamber data (Fisher et al., 1991) were fairly accurate. Unfortunately, no additional closed chamber data were available. In addition, the discrepancies in observed and predicted TCE blood concentrations following inhalation exposures remain unresolved. Hypothesized explanations such as fractional uptake or presystemic elimination could have a substantial impact on estimates of total metabolism.

In addition, no data are directly informative as to the fraction of total metabolism in the lung, the amount of "untracked" hepatic oxidative metabolism (parameterized as "FracDCA"), or any other extrahepatic metabolism. The lung metabolism as currently modeled could just as well be located in other extrahepatic tissues, with little change in calibration. In addition, it is difficult to distinguish between untracked hepatic oxidative metabolism and GSH conjugation, particularly at low doses.

A.2.3.2. *Rat Model*

A.2.3.2.1. ***Group-specific and population-based predictions.*** As with the mouse mode, initially, the sampled group-specific parameters were used to generate predictions for comparison to the calibration data. Because these parameters were "optimized" for each group, these "group-specific" predictions should be accurate by design, and indeed they were, as discussed in more detail in Table A-2.

Next, as with the mouse, only samples of the population parameters (means and variances) were used, and "new groups" were sampled from appropriate distribution using these population means and variances. These "new groups" then represent the predicted population distribution, incorporating both variability in the population as well as uncertainty in the population means and variances. These "population-based" predictions were then compared to both the data used in calibration, as well as the additional data identified that was not used in calibration. The Hack et al. (2006) PBPK model used for prediction was modified to accommodate some of the different outputs (e.g., tissue concentrations) and exposure routes (i.v., intra-arterial [i.a.], and intraperivenous [p.v.]) used in the "noncalibration" data, but otherwise unchanged.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in rats

Reference	Simulation #	Calibration data	Discussion
Andersen et al., 1987	7−11	√	Good posterior fits were obtained for these data—closed chamber data with initial concentrations from 100 to 4,640 ppm.
Barton et al., 1995	17−20		It was assumed that the closed chamber volume was the same as for Andersen et al. (1987). However, the initial chamber concentrations are not clear in the paper. The values that were used in the simulations do not appear to be correct, since in many cases the time-course is inaccurately predicted even at the earliest time-points. Conclusions as to these data need to await definitive values for the initial chamber concentrations, which were not available.
Bernauer et al., 1996	1−3	√	Urinary time-course data (Fig 6-7) for TCA, TCOG, and NAcDCVC was given in concentration units (mg/mg creat-h), whereas total excretion at 48 h (Table 2) was given in molar units (mmol excreted). In the original calibration files, the conversion from concentration to cumulative excretion was not consistent-i.e., the amount excreted at 48 h was different. The data were revised using a conversion that forced consistency. One concern, however, is that this conversion amounts to 6.2 mg creatinine over 48 h, or 1.14 micromol/h. This seems very low for rats; Trevisan et al. (2001), in samples from 195 male control rats, found a median value of 4.95 micromol/h, a mean of 5.39 micromol/h, and a 1−99 percentile range of 2.56−10.46 micromol/h. In addition, the NAcDCVC data were revised in include both 1,2- and 2,2-isomers, since the goal of the GSH pathway is primarily to constrain the total flux. Furthermore, because of the extensive interorgan processing of GSH conjugates, and the fact that excretion was still ongoing at the end of the study (48 h), the amount of NAcDCVC recovered can only be a lower bound on the amount ultimately excreted in urine. However, the model does not attempt to represent the excretion time-course of GSH conjugates—it merely models the total flux. This is evinced by the fact that the model predicts complete excretion by the first time point of 12 h, whereas in the data, there is still substantial excretion occurring at 48 h. Posterior fits to these data were poor in all cases except urinary TCA at the highest dose. In all other cases, TCOH/TCOG and TCA excretion was substantially overpredicted, though this is due to the revision of the data (i.e., the different assumptions about creatinine excretion). Unfortunately, of the original calibration data, this is the only one with TCA and TCOH/TCOG urinary excretion. Therefore, that part of the model is poorly calibrated. On the other hand, NAcDCVC was underpredicted for a number of reasons, as noted above. Because of the incomplete capture of NAcDCVC in urine, unless the model can accurately portray the time-course of NAcDCVC in urine, it should probably not be used for calibration of the GSH pathway, except perhaps as a lower bound.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Birner et al., 1993	21−22		These data only showed urine concentrations, so a conversion was made to cumulative excretion based on an assumed urine flow rate of 22.5 mL/d. Based on this, urinary NAcDCVC was underestimated by 100- to 1,000-fold. Urinary TCA was underestimated by about 2-fold in females (barely within the 95% confidence interval), and was accurately estimated in males. Note that data on urinary flow rate from Trevisan et al. (2001) in samples from 195 male control rats showed high variability, with a geometric standard deviation of 1.75, so this may explain the discrepancy in urinary TCA. However, the underestimation of urinary NAcDCVC cannot be explained this way.
Dallas et al., 1991	23−24		At the lower (50 ppm) exposure, arterial blood concentrations were consistently overpredicted by about 2.5-fold, while at the higher (500 ppm) exposure, arterial blood was overpredicted by 1.5- to 2-fold, but within the range of variability. Exhaled breath concentrations were in the middle of the predicted range of variability at both exposure levels. The ratio of exhaled breath and arterial blood should depend largely on the blood-air partition coefficient, with minor dependence on the assumed dead space. This suggests the possibility of some unaccounted-for variability in the partition coefficient (e.g., posterior mean estimated to be 15.7; *in vitro* measured values from the literature are as follows: 25.82 [Sato et al., 1977], 21.9 [Gargas et al., 1989], 25.8 [Koizumi, 1989], 13.2 [Fisher et al., 1989], posterior). Alternatively, there may be a systematic error in these data, since, as discussed below, the fit of the model to the arterial blood data of Keys et al. (2003) was highly accurate.
Fisher et al., 1989	25−28		Good posterior fits were obtained for these data (in females)—closed chamber data with initial concentrations from 300 to 5,100 ppm. There was some slight overprediction of chamber concentrations (i.e., data showed more uptake/metabolism) at the lower doses, but still within the 95% confidence interval.
Fisher et al., 1991	4−6	√	Good posterior fits were obtained from these data—plasma levels of TCA and venous blood levels of TCE.
Green and Prout, 1985	29−30		In naive rats at 500 mg/kg, urinary excretion of TCOH/TCOG and TCA at 24 h was underpredicted (2-fold), although within the 95% confidence interval. With bile-cannulated rats at the same dose, the amount of TCOG in bile was well within the 95% confidence interval. Urinary TCOH/TCOG was still underpredicted by about 2-fold, but again still within the 95% confidence interval.
Jakobson et al., 1986	31		The only data from the experiment (500 ppm in female rats) were venous blood concentrations during exposure. There were somewhat overpredicted at early times (outside of 95% confidence interval for first 30 min) but was well predicted at the termination of exposure. This suggests some discrepancies in uptake to tissues that reach equilibrium quickly—the model approaches the peak concentration at a faster rate than the data suggest.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Kaneko et al., 1994	32−35		In these inhalation experiments (50−1,000 ppm), urinary excretion of TCOH/TCOG and TCA are consistently overpredicted, particularly at lower doses. The discrepancy decreases systematically as dose increases, with TCA excretion accurately predicted at 1,000 ppm (TCOH/TCOG excretion slightly below near the lower 95% confidence interval at this dose). This suggests a discrepancy in the dose-dependence of TCOH, TCOG, and TCA formation and excretion. On the other hand, venous blood TCE concentrations postexposure are well predicted. TCE blood concentrations right at the end of the exposure are overpredicted; however, concentrations are rapidly declining at this point, so even a few minutes delay in obtaining the blood sample could explain the discrepancy.
Keys et al., 2003	36−39		These experiments collected extensive data on TCE in blood and tissues following i.a., oral, and inhalation exposures. For the i.a. exposure, blood and tissue concentrations were very well predicted by the model, even with the use of the rapidly perfused tissue concentration as a surrogate for brain, heart, kidney, liver, lung, and spleen concentrations. Similarly accurate predictions were found with the higher (500 ppm) inhalation exposure. At the lower inhalation exposure (50 ppm), there was some minor overprediction of concentrations (2-fold), particularly in fat, but values were still within the 95% confidence intervals. For oral exposure, the GI absorption parameters needed to be revised substantially to obtain a good fit. When the values reported by Keys et al. (2003) were used, the model generally had accurate predictions. Two exceptions were the values in the gut and fat in the first 30 min after exposure. In addition, the liver concentration was over-predicted in the first 30 min, and under-predicted at 2−4 h, but still within the 95% confidence interval during the entire period.
Kimmerle and Eben, 1973a	40−44		In these inhalation experiments (49 to 3,160 ppm), urinary excretion of TCOH/TCOG was systematically overpredicted (>2-fold; outside 95% confidence interval), while excretion of TCA was accurately predicted. In addition, elimination by exhaled breath was substantially overpredicted at the lowest exposure. Blood TCOH levels were accurately predicted, but blood TCE levels were overpredicted at the 55 ppm. Part of the discrepancies may be due to limited analytic sensitivities at the lower exposures.
Larson and Bull, 1992b	12−14	√	The digitization in the calibration file did not appear to be accurate, as there was a 10-fold discrepancy with the original paper in the TCOH data. The data were replaced this those used by Clewell et al. (2000) and Bois (2000b). Except for the TCOH data, differences between the digitizations were 20% or less. Adequate posterior predictions were obtained for these data (oral dosing from 200 mg/kg to 3,000 mg/kg). All predictions were within the 95% confidence interval of posterior predictions. Better fits were obtained using group-specific posterior parameters, for which gut absorption and TCA urinary excretion parameters were more highly identified.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Lash et al., 2006	45−46		In these corn-oil gavage experiments, almost all of the measurements appeared to be systematically low, sometimes by many orders of magnitude. For example, at the lowest dose (263 mg/kg), urinary excretion of TCOH/TCOG and TCA, and blood concentrations of TCOH were overpredicted by the model by around >10^5-fold. TCE concentrations in blood and tissues at 2, 4, and 8 h were underpredicted by 10^3- to 10^4-fold. Many studies, including those using the corn oil gavage (Green and Prout, 1985; Hissink et al., 2002), with similar ranges of oral doses show good agreement with the model, it seems likely that these data are aberrant.
Lee et al., 1996	47−61		This extensive set of experiments involved multiroute administration of TCE (oral, i.v., i.a., or portal vein), with serial measurements of arterial blood concentrations. For the oral route (8 mg/kg−64 mg/kg), the GI absorption parameters had to be modified. The values from Keys et al. (2003) were used, and the resulting predictions were quite accurate, albeit a more prominent peak was predicted. Predictions >30 min after dosing were highly accurate. For the i.v. route (0.71 mg/kg−64 mg/kg), predictions were also highly accurate in almost all cases. At the lower doses (0.71 mg/kg and 2 mg/kg), there was slight overprediction in the first 30 min after dosing. At highest dose (64 mg/kg), there was slight underprediction between 1 and 2 h after dosing. In all cases, the values were within the 95% confidence interval. For the i.a. route (0.71 mg/kg−16 mg/kg), all predictions were very accurate. For the p.v. route (0.71 mg/kg−64 mg/kg), predictions still remained in the 95% confidence interval, although there was more variation. At the lowest dose, there was overprediction in the first 30 min after dosing. At the highest two doses (16 mg/kg and 64 mg/kg), there was slight underprediction between 1 and 5 h after dosing. This may in part be because a pharmacodynamic change in metabolism (e.g., via direct solvent injury proposed by Lee et al., 2000).
Lee et al., 2000	62−69		In the p.v. and i.v. exposures, blood and liver concentrations were accurately predicted. For oral exposures, the GI absorption parameters needed to be changed. While the values from Keys et al. (2003) led to accurate predictions for lower doses (2 mg/kg−16 mg/kg), at the higher doses (48 mg/kg−432 mg/kg), much slower absorption was evident. Comparisons at these higher dose are not meaningful without calibration of absorption parameters.
Prout et al., 1985	15	√	Adequate posterior fits were obtained for these data—rat dosing at 1,000 mg/kg in corn oil. All predictions were within the 95% confidence interval of posterior predictions. Better fits were obtained using group-specific posterior parameters, for which gut absorption and TCA urinary excretion parameters were more highly identified.

Table A-2. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in rats (continued)

Reference	Simulation #	Calibration data	Discussion
Stenner et al., 1997	70		As with other oral exposures, different GI absorption parameters were necessary. Again, the values from Keys et al. (2003) were used, with some success. Blood TCA levels were accurately predicted, while TCOH blood levels were systematically under-predicted (up to 10-fold). Additional data with TCOH and TCA dosing, including naive and bile-cannulated rats, can be added when those exposure routes are added to the model. These could be useful in better calibrating the enterohepatic recirculation parameters.
Templin et al., 1995	16	√	Adequate posterior fits were obtained for blood TCA from these data—oral dosing at 100 mg/kg in Tween. Blood levels of TCOH were underpredicted, while the time-course of TCE in blood exhibited an earlier peak. Better fits were obtained using group-specific posterior parameters, for which gut absorption and TCA urinary excretion parameters (and to a lesser extent glucuronidation of TCOH and biliary excretion of TCOG) were more highly identified.

GI = gastrointestinal, NAc-1,2-DCVC = N-acetyl-S-(1,2-dichlrovinyl)-L-cysteine, NAc-2,2-DCVC = N-acetyl-S-(2,2-dichlrovinyl)-L-cysteine, NAcDCVC = NAc-1,2-DCVC and NAc-2,2-DCVC.

A.2.3.2.1.1. ***<u>Group-specific predictions and calibration data</u>*****.** [See Appendix.linked.files\AppA.2.3.2.1.1.Hack.rat.group.calib.TCE.DRAFT.pdf.]

A.2.3.2.1.2. ***<u>Population-based predictions and calibration and additional evaluation data</u>*****.** [See Appendix.linked.files\AppA.2.3.2.1.2.Hack.rat.pop.calib.eval.TCE.DRAFT.pdf.]

A.2.3.2.2. ***Conclusions regarding rat model.***

A.2.3.2.2.1. ***<u>Trichloroethylene (TCE) concentrations in blood and tissues generally well-predicted</u>*****.** The PBPK model for the parent compound appears to be robust. Multiple datasets not used for calibration with TCE measurements in blood and tissues were simulated, and overall the model gave very accurate predictions. A few datasets seemed somewhat anomalous—Dallas et al. (1991), Kimmerle and Eben (1973a), Lash et al. (2006). However, data from Kaneko et al. (1994), Keys et al. (2003), and Lee et al. (1996, 2000) were all well simulated, and corroborated the data used for calibration (Fisher et al., 1991; Larson and Bull, 1992b; Prout et al., 1985; Templin et al., 1995). Particularly important is the fact that tissue concentrations from Keys et al. (2003) were well simulated.

A.2.3.2.2.2. ***<u>Total metabolism probably well simulated, but ultimate disposition is less certain</u>*****.** Closed chamber data are generally thought to provide a good indicator of total metabolism. Two closed chamber studies not used for calibration were available—Barton et al. (1995) and Fisher et al. (1989). Additional experimental information is required to analyze the Barton et al. (1995) data, but the predictions for the Fisher et al. (1989) data were quite accurate.

However, the ultimate disposition of metabolized TCE is much less certain. Clearly, the flux through the GSH pathway is not well constrained, with apparent discrepancies between the N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (NAc-1,2-DCVC) data of Bernauer et al. (1996) and Birner et al. (1993). Moreover, each of these data has limitations—in particular, the Bernauer et al. (1996) data show that excretion is still substantial at the end of the reporting period, so that the total flux of mercapturates has not been collected. Moreover, there is some question as to the consistency of the Bernauer et al. (1996) data (Table 2 vs. Figures 6 and 7), since a direct comparison seems to imply a very low creatinine excretion rate. The Birner et al. (1993) data only report concentrations—not total excretion—so a urinary flow rate needs to be assumed.

In addition, no data are directly informative as to the fraction of total metabolism in the lung or the amount of "untracked" hepatic oxidative metabolism (parameterized as "FracDCA"). The lung metabolism could just as well be located in other extrahepatic tissues, with little change in calibration. In addition, there is a degeneracy between untracked hepatic oxidative metabolism and GSH conjugation, particularly at low doses.

The ultimate disposition of TCE as excreted TCOH/TCOG or TCA is also poorly estimated in some cases, as discussed in more detail below.

A.2.3.2.2.3. ***Trichloroethanol−trichlorethanol-glucuronide conjugate (TCOH/TCOG) submodel requires revision and recalibration*****.** TCOH blood levels of TCOH were inconsistently predicted in noncalibration datasets (well predicted for Larson and Bull [1992b]; Kimmerle and Eben [1973a]; but not Stenner et al. [1997] or Lash et al. [2006]), and the amount of TCE ultimately excreted as TCOG/TCOH also appeared to be poorly predicted. The model generally underpredicted TCOG/TCOH urinary excretion (underpredicted Green and Prout [1985], overpredicted Kaneko et al. [1994], Kimmerle and Eben [1973a], and Lash et al. [2006]). This may in part be due to discrepancies in the Bernauer et al. (1996) data as to the conversion of excretion relative to creatinine.

Moreover, there are relatively sparse data on TCOH in combination with a relatively complex model, so the identifiability of various pathways—conversion to TCA, enterohepatic recirculation, and excretion in urine—is questionable.

This could be improved by the ability to incorporate TCOH dosing data from Merdink et al. (1999) and Stenner et al. (1997), the latter of which included bile duct cannulation to better estimate enterohepatic recirculation parameters. However, the TCOH dosing in these studies is by the intravenous route, whereas with TCE dosing, TCOH first appears in the liver. Thus, the model needs to ensure that any first pass effect is accounted for appropriately. Importantly, the estimated clearance rate for glucuronidation of TCOH is substantially greater than hepatic blood flow. That is, since TCOH is formed in the liver from TCE, and TCOH is also glucuronidated in the liver to TCOG, a substantial portion of the TCOH may be glucuronidated before reaching systemic circulation. Thus, suggests that a liver compartment for TCOH is necessary. Furthermore, because substantial TCOG can be excreted in bile from the liver prior to systemic circulation, a liver compartment for TCOG may also be necessary to address that first pass effect.

The addition of the liver compartment will necessitate several changes to model parameters. The distribution volume for TCOH will be replaced by two parameters: the liver:blood and body:blood partition coefficients. Similarly for TCOG, liver:blood and body:blood partition coefficients will need to be added. Clearance of TCOH to TCA and TCOG can be redefined as occurring in the liver, and urinary clearance can be redefined as coming from the rest of the body.

Finally, additional clearance of TCOH (not to TCA or urine—e.g., to DCA or some other untracked metabolite) is possible. This may in part explain the discrepancy between the accurate predictions to blood data along with poor predictions to urinary excretion (i.e., there is a missing pathway). This pathway can be considered for inclusion, and limits can be placed on it using the available data.

A.2.3.2.2.4. ***Trichloroacetic acid (TCA) submodel would benefit from revised trichloroethanol/trichloroethanol-glucuronide conjugate (TCOH/TCOG) submodel and incorporating TCA dosing studies*.** While blood levels of TCA were well predicted in the one noncalibration dataset (Stenner et al., 1997), the urinary excretion of TCA was inconsistently predicted (underpredicted in Green and Prout [1985]; overpredicted in Kaneko et al. [1994] and Lash et al. [2006]; accurately predicted in Kimmerle and Eben [1973a]). Because TCA is in part derived from TCOH, a more accurate TCOH/TCOG submodel would probably improve the TCA submodel.

In addition, there are a number of TCA dosing studies that could be used to isolate the TCA kinetics from the complexities of TCE and TCOH. These could be readily incorporated into the TCA submodel.

Finally, as with TCOH, additional clearance of TCA (not to urine—e.g., to DCA or some other untracked metabolite) is possible. This may in part explain the discrepancy between the accurate predictions to blood data along with poor predictions to urinary excretion (i.e., there is a missing pathway). As with TCOH, this pathway can be considered for inclusion, and limits can be placed on it using the available data.

A.2.3.3. ***Human model.***

A.2.3.3.1. ***Individual-specific and population-based predictions.*** As with the mouse and rat models, initially, the sampled individual-specific parameters (the term “individual” instead of “group” is used since human variability was at the individual level) were used to generate predictions for comparison to the calibration data. Because these parameters were “optimized” for each individual, these “individual-specific” predictions should be accurate by design. However, unlike for the rat, this was not the case for some experiments (this is partially responsible for the slower convergence), although the inaccuracies were generally less than those in the mouse. For example, alveolar air concentrations were systematically overpredicted for several datasets. There was also variability in the ability to predict the precise time-course of TCA and TCOH blood levels, with a few datasets more difficult for the model to accommodate. These data are discussed further in Table A-3.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in humans

Reference	Simulation #	Calibration data	Discussion
Bartonicek, 1962	38−45		The measured minute-volume was multiplied by a factor of 0.7 to obtain an estimate for alveolar ventilation rate, which was fixed for each individual. These data are difficult to interpret because they consist of many single data points. It is easiest to go through the measurements one at a time: *Alveolar retention* (1—exhaled dose/inhaled dose during exposure) and *Retained dose* (inhaled dose—exhaled dose during exposure): Curiously, retention was generally under-predicted, which in many cases retained dose was accurately predicted. However, alveolar retention was an adjustment of the observed total retention: TotRet = (CInh – CExh)/CInh = QAlv × (CInh – CAlv)/(MV × CInh), so that AlvRet = TotRet × (QAlv/MV), with QAlv/MV assumed to be 0.7 Because retained dose is the more relevant quantity, and is less sensitive to assumptions about QAlv/MV, then this is the better quantity to use for calibration. *Urinary TCOG*: This was generally underpredicted, although generally within the 95% confidence interval. Thus, these data will be informative as to interindividual variability. *Urinary TCA*: Total collection (at 528 h) was accurately predicted, although the amount collected at 72 h was generally under-predicted, sometimes substantially so. *Plasma TCA*: Generally well predicted.
Bernauer et al., 1996	1−3	√	Individual-specific predictions were good for the time-courses of urinary TCOG and TCA, but poor for total urinary TCOG+TCA and for urinary NAc-1,2-DCVC. One reason for the discrepancy in urinary excretion of TCA and TCOG is that the urinary time-course data (see Figures 4-5 in the manuscript) for TCA, TCOG, and NAc-1,2-DCVC was given in concentration units (mg/mg creat-h), whereas total excretion at 48 h (Table 2 in the manuscript) was given in molar units (mmol excreted). In the original calibration files, the conversion from concentration to cumulative excretion was not consistent—i.e., the amount excreted at 48 h was different. For population-based predictions, the data were revised using a conversion that forced consistency. One concern, however, is that this conversion amounts to 400−500 mg creatinine over 48 h, or 200−250 mg/d, which seems rather low. For instance, Araki (1978) reported creatinine excretion of 11.5+/-1.8 mmol/24 h (mean +/- SD) in 9 individuals, corresponding to 1,300 +/- 200 mg/d. In addition, for population-based predictions, the data were revised include both the NAc-1,2-DCVC and the N acetyl-S-(2,2-dichlorovinyl)-L-cysteine isomer (the combination denoted NAcDCVC), since the goal of the GSH pathway is primarily to constrain the total flux. Furthermore, because of the extensive interorgan processing of GSH conjugates, and the fact that excretion was still ongoing at the end of the study (48 h), the amount of NAcDCVC recovered can only be a lower bound on the amount ultimately excreted in urine. However, the model does not attempt to represent the excretion time-course of GSH conjugates—it merely models the total flux. This is evinced by the fact that the model predicts complete excretion by the first time point of 12 h, whereas in the data, there is still substantial excretion occurring at 48 h.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in humans (continued)

Reference	Simulation #	Calibration data	Discussion
Bernauer et al., 1996 (continued)	1−3 (continued)		Population-based posterior fits to these data were quite good for urinary TCA and TCOH, but not for NAcDCVC in urine. Because of the incomplete capture of NAcDCVC in urine, unless the model can accurately portray the time-course of NAcDCVC in urine, it should probably not be used for calibration of the GSH pathway, except perhaps as a lower bound.
Bloemen et al., 2001	72−75		Like Bartonicek (1962), these data are more difficult to interpret due to their being single data points for each individual and exposure. However, in general, posterior population-based estimates of retained dose, urinary TCOG, and urinary TCA were fairly accurate, staying within the 95% confidence interval, and mostly inside the interquartile range. The data on GSH mercapturates are limited—first they are all nondetects. In addition, because of the 48−56 h collection period, excretion of GSH mercapturates is probably incomplete, as noted above in the discussion of Bernauer et al. (1996).
Chiu et al., 2007	66−71		The measured minute-volume was multiplied by a factor of 0.7 to obtain an estimate for alveolar ventilation rate, which was fixed for each individual. Alveolar air concentrations of TCE were generally well predicted, especially during the exposure period. Postexposure, the initial drop in TCE concentration was generally further than predicted, but the slope of the terminal phase was similar. Blood concentrations of TCE were consistently overpredicted for all subjects and occasions. Blood concentrations of TCA were consistently over-predicted, though mostly staying in the lower 95% confidence region. Blood TCOH (free) levels were generally over-predicted, in many cases falling below the 95% confidence region, though in some cases the predictions were accurate. On the other hand, total TCOH (free+glucuronidated) was well predicted (or even under-predicted) in most cases—in the cases where free TCOH was accurately predicted, total TCOH was underpredicted. The free and total TCOH data reflect the higher fraction of TCOH as TCOG than previously reported (e.g., Fisher et al. [1998] reported no detectable TCOG in blood). Data on urinary TCA and TCOG were complicated by some measurements being saturated, as well as the intermittent nature of urine collection after Day 3. Thus, only the nonsaturated measurements for which the time since the last voiding was known were included for direct comparison to the model predictions. Saturated measurements were kept track of separately for comparison, but were considered only rough lower bounds. TCA excretion was generally over-predicted, whether looking at unsaturated or saturated measurements (the latter, would of course, be expected). Urinary excretion of TCOG generally stayed within the 95% confidence range.
Fernandez et al., 1977			Alveolar air concentrations are somewhat overestimated. Other measurements are fairly well predicted.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in humans (continued)

Reference	Simulation #	Calibration data	Discussion
Fisher et al., 1998	13−33	√	The majority of the data used in the calibration (both in terms of experiments and data points) came from this study. In general, the individual-specific fits to these data were good, with the exception of alveolar air concentrations, which were consistently over-predicted. In addition, for some individuals, the shape of the TCOH time-course deviated from the predictions (#14, #24, #29, and #30)—the predicted peak was too "sharp," with underprediction at early times. Simulation #23 showed the most deviation from predictions, with substantial inaccuracies in blood TCA, TCOH, and urinary TCA. Interestingly, in the population-based predictions, in same cases the predictions were not very accurate—indicating that the full range of population variability is not accounted for in the posterior simulations. This is particularly the case with venous blood TCE concentrations, which are generally under-predicted in population estimates (although in some cases the predictions are accurate). One issue with the way in which these data were utilized in the calibration is that in some cases, the same individual was exposed to two different concentrations, but in the calibration, they were treated as separate "individuals." Thus, parameters were allowed to vary between exposures, mixing interindividual and interoccasion variability. It is recommended that in subsequent calibrations, the different occasions with the same individual be modeled together. This will also allow identification of any dose-related changes in parameters (e.g., saturation).
Kimmerle and Eben, 1973b	46−57		Blood TCE levels are generally over-predicted for both single and multiexposure experiments. However, levels at the end of exposure are rapidly changing, so some of those values may be better predicted if the "exact" time after cessation of exposure were known. Blood TCOH levels are fairly accurately predicted, although in some individuals in single exposure experiments, there is a tendency to overpredict at early times and underpredict at late times. In multiexposure experiments, the decline after the last exposure was somewhat steeper than predicted. Urinary excretion of TCA and TCOH was well predicted. Only grouped data on alveolar air concentrations were available, so they were not used.
Laparé et al., 1995	34	√	Predictions for these data were not accurate. However, there was an error in some of the exposure concentrations used in the original calibration. In addition, the last exposure "occasion" in these experiments involved exercise/workload, and so should be excluded. Finally, individual data are available for these experiments.
	62−65 (individual data)		Taking into account these changes, population-based predictions were somewhat more accurate. However, alveolar air concentrations and venous blood TCE concentrations were still over-predicted.

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in humans (continued)

Reference	Simulation #	Calibration data	Discussion
Monster et al., 1976	5−6 (summary data)	√	Individual-specific predictions were quite good, except that for blood TCA concentrations exhibited a higher peak that predicted. However, TCOH values were entered as free TCOH, whereas the TCOH data were actually total (free+glucuronidated) TCOH. Therefore, for population-based predictions, this change was made. In addition, as with the Monster et al. (1979) data, minute-volume and exhaled air concentrations were measured and incorporated for population-based predictions. Finally, individual-specific data are available, so in those data should replace the grouped data in any revised calibration. These individual data also included estimates of retained dose based on complete inhaled and exhaled air samples during exposure. For population-based predictions, as with the Monster et al. (1979) data, grouped urinary and blood TCOH/TCOG was somewhat under-predicted in the population-based predictions, and grouped alveolar and blood TCE concentrations were somewhat over-predicted.
	58−61 (individual data)		The results for the individual data were similar, but exhibited substantially greater variability that predicted. For instance, in subject A, blood TCOH levels were generally greater than the 95% confidence interval at both 70 and 140 ppm, whereas predictions for blood TCOH in subject D were quite good. In another example, for blood TCE levels, predictions for subject B were quite good, but those for subject D were poor (substantially overpredicted). Thus, it is anticipated that adding these individual data will be substantially informative as to interindividual variability, especially since all 4 individuals were exposed at 2 different doses.
Monster et al., 1979	4	√	Individual-specific predictions for these data were quite good. However, TCA values were entered as plasma, whereas the TCA data were actually in whole blood. Therefore, for population-based predictions, this change was made. In addition, two additional time-courses were available that were not used in calibration: exhaled air concentrations and total TCOH blood concentrations. These were added for population-based predictions. In addition, the original article had data on ventilation rate, which as incorporated into the model. The minute volume needed to be converted to alveolar ventilation rate for the model, but this required adjusted for an extra dead space volume of 0.15 L due to use of a mask, as suggested in the article. The measured mean minute volume was 11 L/min, and with a breathing rate of 14 breaths/min (assumed in the article), this corresponding to a total volume of 0.79 L. Subtracting the 0.15 L of mask dead space and 0.15 L of physiological dead space (suggested in the article) gives 0.49 L of total physiological dead space. Thus, the minute volume of 11 L/min was adjusted by the factor 0.49/0.79 to give an alveolar ventilation rate of 6.8 L/min, which is a reasonably typical value at rest. Due to extra nonphysiological dead space issue, some adjustment to the exhaled air predictions also needed to be made. The alveolar air concentration CAlv was, therefore, estimated based on the formula

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in humans (continued)

Reference	Simulation #	Calibration data	Discussion
Monster et al., 1979 (continued)	4 (continued)		CAlv = (CExh × VTot – CInh × VDs)/VAlv where CExh is the measured exhaled air concentration, VTot is the total volume (alveolar space VAlv of 0.49 L, physiological dead space of 0.15 L, and mask dead space of 0.15 L), VDs is the total dead space of 0.3 L, and CInh is the inhaled concentration. Population-based predictions for these data lead to slight underestimation urinary TCOG and blood TCOH levels, as well as some over-prediction of alveolar air and venous blood concentrations by factors of 3~10-fold.
Muller et al., 1972, 1974, 1975	7−10	√	Individual-specific predictions for these data were good, except for alveolar air concentrations. However, several problems were found with these data as utilized in the original calibration: • Digitization problems, particular with the time axis in the multiday exposure study (Simulation 9) that led to measurements taken prior to an exposure modeled as occurring during the exposure. The original digitization from Bois (2000b) and Clewell et al. (2000) was used for population-based estimates. • Original article showed TCA as measured in plasma, not blood as was assumed in the calibration. • Blood was taken from the earlobe, which is thought to be indicative of arterial blood concentrations, rather than venous blood concentrations. • TCOH in blood was free, not total, as Ertle et al. (1972 [cited in Methods]) had no use of beta-glucuronidase in analyzing blood samples. Separate free and total measurements were done in plasma (not whole blood), but these data were not included. • Simulation 9, contiguous data on urinary excretion were only available out to 6 d, so only that data should be included. • Simulation 10, is actually the same as the first day of simulation 9, from Muller et al. (1972, 1975) (the data were reported in both papers), and, thus, should be deleted. These were corrected in the population-based estimates. Alveolar air concentration measurements remained over-predicted, while the change to arterial blood led to over-prediction of those measurements during exposure (but postexposure predictions were accurate).

Table A-3. Evaluation of Hack et al. (2006) PBPK model predictions for *in vivo* data in humans (continued)

Reference	Simulation #	Calibration data	Discussion
Muller et al., 1974	81−82 (TCA and TCOH dosing)		The experiment with TCA showed somewhat more rapid decline in plasma levels than predicted, but still well within the 95% confidence range. Urinary excretion was well predicted, but only accounted for 60% of the administered dose—this is not consistent with the rapid decline in TCA plasma levels (10-fold lower than peak at the end of exposure), which would seem to suggest the majority of TCA has been eliminated. With TCOH dosing, blood levels of TCOH were over-predicted in the first 5 hours, perhaps due to slower oral absorption (the augmented model used instantaneous and complete absorption). TCA plasma and urinary excretion levels were fairly well predicted. However, urinary excretion of TCOG was near the bottom of the 95% confidence interval; while, in the same individuals with TCE dosing (Simulation 7), urinary excretion of TCOG was substantially greater (near slightly above the interquartile region). Furthermore, total TCA and TCOG urinary excretion accounted for <40% of the administered dose.
Paycok and Powell, 1945	35−37		Population-based fits were good, within the inner quartile region.
Sato et al., 1977	76		Both alveolar air and blood concentrations are over-predicted in this model. Urinary TCA and TCOG, on the other hand, are well predicted.
Stewart et al., 1970	11	√	Individual-specific predictions for these data were good, except for some alveolar air concentrations. However, a couple of problems were found with these data as utilized in the original calibration: • The original article noted that individual took a lunch break during which there was no exposure. This was not accounted for in the calibration runs, which a assumed a continuous 7-h exposure. The exposures were, therefore, revised with a 3-h morning exposure (9−12), a 1 hour lunch break (12−1), and 4-h afternoon exposure (1−5), to mimic a typical workday. The times of the measurements had to be revised as well, since the article gave "relative" rather than "absolute" times (e.g., x hours postexposure). • Contiguous data on urinary excretion were only available out to 11 d, so only that data should be included (Table 2). With these changes, population-based predictions of urinary TCA and TCOG were still accurate, but alveolar air concentrations were over-predicted.
Triebig et al., 1976	12	√	Only two data points are available for alveolar air, and blood TCA and TCOH. Only one data point is available on blood TCE. Alveolar air was underpredicted at 24 h. Blood TCA and TCOH were within the 95% confidence ranges. Blood TCE was over-predicted substantially (outside 95% confidence range).

SD = standard deviation.

Next, only samples of the population parameters (means and variances) were used, and "new individuals" were sampled from appropriate distribution using these population means and variances. These "new individuals" then represent the predicted population distribution, incorporating both variability as well as uncertainty in the population means and variances. These "population-based" predictions were then compared to both the data used in calibration, as well as the additional data identified that was not used in calibration. The Hack et al. (2006) PBPK model was modified to accommodate some of the different outputs (e.g., arterial blood, intermittently collected urine, retained dose) and exposure routes (TCA i.v., oral TCA, and TCOH) used in the "noncalibration" data, but otherwise unchanged.

A.2.3.3.1.1. ***Individual-specific predictions and calibration data*.** [See Appendix.linked.files\AppA.2.3.3.1.1.Hack.human.indiv.calib.TCE.DRAFT.pdf.]

A.2.3.3.1.2. ***Population-based predictions and calibration and additional evaluation data*.** [See Appendix.linked.files\AppA.2.3.3.1.2.Hack.human.pop.calib.eval.TCE.DRAFT.pdf.]

A.2.3.3.2. ***Conclusions regarding human model.***

A.2.3.3.2.1. ***Trichloroethylene (TCE) concentrations in blood and air are often not well-predicted*.** Except for the Chiu et al. (2007) during exposure, TCE alveolar air levels were consistently overpredicted. Even in Chiu et al. (2007), TCE levels postexposure were over-predicted, as the drop-off after the end of exposure was further than predicted. Because predictions for retained dose appear to be fairly accurate, this implies that less clearance is occurring via exhalation than predicted by the model. This could be the result of additional metabolism or storage not accounted for by the model.

Except for the Fisher et al. (1998) data, TCE blood levels were consistently overpredicted. Because the majority of the data used for calibration was from Fisher et al. (1998), this implies that the Fisher et al. (1998) data had blood concentrations that were consistently higher than the other studies. This could be due to differences in metabolism and/or distribution among studies.

Interestingly, the mouse inhalation data also exhibited inaccurate prediction of blood TCE levels. Hypotheses such as fractional uptake or presystemic elimination due to local metabolism in the lung have not been tested experimentally, nor is it clear that they can explain the discrepancies.

Due to the difficulty in accurately predicted blood and air concentrations, there may be substantial uncertainty in tissue concentrations of TCE. However, such potential model errors can be characterized estimated and estimated as part of a revised calibration.

A.2.3.3.2.2. *Trichloroacetic acid (TCA) blood concentrations well predicted following trichloroethylene (TCE) exposures, but some uncertainty in TCA flux and disposition*. TCA blood and plasma concentrations and urinary excretion, following TCE exposure, are generally well predicted. Even though the model's central estimates over-predicted the Chiu et al. (2007) TCA data, the confidence intervals were still wide enough to encompass those data.

However, the total flux of TCA may not be correct, as evidenced by TCA dosing studies, none of which were included in the calibration. In these studies, total recovery of urinary TCA was found to be substantially less than the administered dose. However, the current model assumes that urinary excretion is the only source of clearance of TCA. This leads to overestimation of urinary excretion. This fact, combined with the observation that under TCE dosing, the model appears to give accurate predictions of TCA urinary excretion for several datasets, strongly suggests a discrepancy in the amount of TCA formed from TCE. That is, since the model appears to overpredict the fraction of TCA that appears in urine, it may be reducing TCA production to compensate. Inclusion of the TCA dosing studies, along with inclusion of a nonrenal clearance pathway, would probably be helpful in reducing these discrepancies. Finally, improvements in the TCOH/TCOG submodel, below, should also help to insure accurate estimates of TCA kinetics.

A.2.3.3.2.3. *Trichloroethanol−trichlorethanol-glucuronide conjugate (TCOH/TCOG) submodel requires revision and recalibration*. Blood levels of TCOH and urinary excretion of TCOG were generally well predicted. Additional individual data show substantial interindividual variability than can be incorporated into the calibration. Several errors as to the measurement of free or total TCOH in blood need to be corrected.

A few inconsistencies with noncalibration datasets stand out. The presence of substantial TCOG in blood in the Chiu et al. (2007) data are not predicted by the model. Interestingly, only two studies that included measurements of TCOG in blood (rather than just total TCOH or just free TCOH)—Muller et al. (1975), which found about 17% of total TCOH to be TCOG, and Fisher et al. (1998), who could not detect TCOG. Both of these studies had exposures at 100 ppm. Interestingly Muller et al. (1975) reported increased TCOG (as fraction of total TCOH) with ethanol consumption, hypothesizing the inhibition of a glucuronyl transferase that slowed glucuronidation. This also would result in a greater half-life for TCOH in blood with ethanol consumptions, which was observed.

An additional concern is the over-prediction of urinary TCOG following TCOH administration from the Muller et al. (1974) data. Like the case of TCA, this indicates that some other source of TCOH clearance (not to TCA or urine—e.g., to DCA or some other untracked metabolite) is possible. This pathway can be considered for inclusion, and limits can be placed on it using the available data.

Also, as for TCA, the fact that blood and urine are relatively well predicted from TCE dosing strongly suggests a discrepancy in the amount of TCOH formed from TCE. That is, since the model appears to overpredict the fraction of TCOH that appears in urine, it may be reducing TCOH production to compensate.

Finally, as with the rat and mice, the model needs to ensure that any first pass effect is accounted for appropriately. Particularly for the Chiu et al. (2007) data, in which substantial TCOG appears in blood, since TCOH is formed in the liver from TCE, and TCOH is also glucuronidated in the liver to TCOG, a substantial portion of the TCOH may be glucuronidated before reaching systemic circulation. Thus, suggests that a liver compartment for TCOH is necessary. Furthermore, because substantial TCOG can be excreted in bile from the liver prior to systemic circulation, a liver compartment for TCOG may also be necessary to address that first pass effect. In addition, in light of the Chiu et al. (2007) data, it may be useful to expand the prior range for the K_M of TCOH glucuronidation.

The addition of the liver compartment will necessitate several changes to model parameters. The distribution volume for TCOH will be replaced by two parameters: the liver:blood and body:blood partition coefficients. Similarly for TCOG, liver:blood and body:blood partition coefficients will need to be added. Clearance of TCOH to TCA and TCOG can be redefined as occurring in the liver, and urinary clearance can be redefined as coming from the rest of the body. Fortunately, there are *in vitro* partition coefficients for TCOH. It may be important to incorporate the fact that Fisher et al. (1998) found no TCOG in blood. This can be included by having the TCOH data be used for both free and total TCOH (particularly since that is how the estimation of TCOG was made—by taking the difference between total and free).

A.2.3.3.2.4. *<u>Uncertainty in estimates of total metabolism</u>*. Estimates of total recovery after TCE exposure (TCE in exhaled air, TCA and TCOG in urine) have been found to be only 60−70% (Monster et al., 1976, 1979; Chiu et al., 2007). Even estimates of total recovery after TCA and TCOH dosing have found 25−50% unaccounted for in urinary excretion (Paycok and Powell, 1945; Muller et al., 1974). Bartonicek found some TCOH and TCA in feces, but this was about 10-fold less than that found in urine, so this cannot account for the discrepancy. Therefore, it is likely that additional metabolism of TCE, TCOH, and/or TCA are occurring. Additional metabolism of TCE could account for the consistent overestimation of TCE in blood and exhaled breath found in many studies. However, no data are *directly* informative as to the fraction of total metabolism in the lung, the amount of "untracked" hepatic oxidative metabolism (parameterized as "FracDCA"), or any other extrahepatic metabolism. The lung (TB) metabolism as currently modeled could just as well be located in other extrahepatic tissues, with little change in calibration. In addition, it is difficult to distinguish between untracked hepatic oxidative metabolism and GSH conjugation, particularly at low doses.

A.3. PRELIMINARY ANALYSIS OF MOUSE GAS UPTAKE DATA: MOTIVATION FOR MODIFICATION OF RESPIRATORY METABOLISM

Potential different model structures can be investigated using the core PBPK model containing averaged input parameters, since this approach saves computational time and is more efficient when testing different structural hypotheses. This approach is particularly helpful for quick comparisons of data with model predictions. During the calibration process, this approach was used for different routes of exposure and across all three species. For both mice and rats, the closed chamber inhalation data resulted in fits that were considered not optimal when visually examined. Although closed chamber inhalation usually combines multiple animals per experiment, and may not be as useful in differentiating between individual and experimental uncertainty (Hack et al., 2006), closed chamber data do describe *in vivo* metabolism and have been historically used to quantify averaged *in vivo* Michaelis-Menten kinetics in rodents.

There are several assumptions used when combining PBPK modeling and closed chamber data to estimate metabolism via regression. The key experimental principles require a tight, sealed, or air-closed system where all chamber variables are controlled to known set points or monitored, that is all except for metabolism. For example, the inhalation chamber is calibrated without an animal, to determine normal absorption to the empty system. This empty chamber calibration is then followed with a dead animal experiment, identical in every way to the *in vivo* exposure, and is meant to account for every factor other than metabolism, which is zero in the dead animal. When the live animal(s) are placed in the chamber, oxygen is provided for, and carbon dioxide accumulated during breathing is removed by absorption with a chemical scrubber. A bolus injection of the parent chemical, TCE, is given and this injection time starts the inhalation exposure. The chemical inside the chamber will decrease with time, as it is absorbed by the system and the metabolic process inside the rodent. Since all known processes contributing to the decline are quantified, except for metabolism, the metabolic parameters can be extracted from the total chamber concentration decline using regression techniques.

The basic structure for the PBPK model that is linked to closed chamber inhalation data has the same basic structure as described before. The one major difference is the inclusion of one additional equation that accounts for mass balance changes inside the inhalation chamber or system, and connects the chamber with the inhaled and exhaled concentrations breathed in and out by the animal:

$$\frac{dA_{Ch}}{dt} = RATS\left(Q_P\right)(C_X - \frac{A_{Ch}}{V_{Ch}}) - K_{LOSS}A_{Ch} \qquad \text{(Eq. A-4)}$$

where

$RATS$	= number of animals in the chamber
Q_P	= alveolar ventilation rate
C_X	= exhaled concentration
A_{Ch}	= net amount of chemical inside chamber
V_{Ch}	= volume of chamber
K_{LOSS}	= loss rate constant to glassware.

An updated model was developed that included updated physiological and chemical-specific parameters as well as GSH metabolism in the liver and kidney, as discussed in Chapter 3. The PBPK model code was translated from MCSim to use in Matlab© (version 7.2.0.232, R2006a, Natick, MA) using their m language. This PBPK model made use of fixed or constant, averaged values for physiological, chemical and other input parameters; there were no statistical distributions attached to each average value. As an additional step in quality control, mass balance was checked for the MCSim code, and comparisons across both sets of code were made to ensure that both sets of predictions were the same.

The resulting simulations were compared to mice gas uptake data (Fisher et al., 1991) after some adjustments of the fat compartment volumes and flows based on visual fits, and limited least-squares optimization of just V_{MAX} (different for males and females) and K_M (same for males and females). The results are shown in the top panels of Figures A-3−A-4, which showed poor fits particularly at lower chamber concentrations. In particular, metabolism is observed to be faster than predicted by simulation. This is directly related to metabolism of TCE being limited by hepatic blood flow at these exposures. Indeed, Fisher et al. (1991) was able to obtain adequate fits to these data by using cardiac output and ventilation rates that were about 2-fold higher than is typical for mice. Although their later publication reporting inhalation experiments (Greenberg et al., 1999) used the lower values from Brown et al. (1997) for these parameters, they did not revisit the Fisher et al. (1991) data with the updated model. In addition, the Hack et al. (2006) model estimated the cardiac output and ventilation rate and for these experiments to be about 2-fold higher than typical. However, it seems unlikely that cardiac output and ventilation rate were really as high as used in these models, since TCE and other solvents typically have central nervous system-depressing effects. In the mouse, after the liver, the lung has the highest rate of oxidative metabolism, as assessed by *in vitro* methods (see footnote in Section 3.5.4.2 for a discussion of why kidney oxidative metabolism is likely to be minor quantitatively). In addition, TCE administered via inhalation is available to the lung directly, as well as through blood flow. Therefore, it was hypothesized that a more refined treatment of respiratory metabolism may be necessary to account for the additional metabolism.

The structure of the updated respiratory metabolism model is shown in Figure A-5, with the mathematical formulation shown in the model code in Section A.6, where the "D" is the diffusion rate, "concentrations" and "amounts" are related by the compartment volume, and the other symbols have their standard meanings in the context of PBPK modeling. In brief, this is a more highly "lumped" version of the Sarangapani et al. (2003) respiratory metabolism model for styrene combined with a "continuous breathing" model to account for a possible wash-in/wash-out effect. In brief, upon inhalation (at a rate equal to the full minute volume, not just the alveolar ventilation), TCE can either (1) diffuse between the respiratory tract lumen and the respiratory tract tissue; (2) remain in the dead space, or (3) enter the gas exchange region. In the respiratory tract tissue, TCE can either be "stored" temporarily until exhalation, during which it diffuses to the "exhalation" respiratory tract lumen, or be metabolized. In the dead space, TCE is transferred directly to the "exhalation" respiratory tract lumen at a rate equal to the minute-volume minus the alveolar ventilation rate, where it mixes with the other sources. In the gas exchange region, it undergoes transfer to and from blood, as is standard for PBPK models of volatile organics. Therefore, if respiratory metabolism is absent (V_{MAX}Clara = 0), then the model reduces to a wash-in/wash-out effect where TCE is temporarily adsorbed to the respiratory tract tissue, the amount of which depends on the diffusion rate, the volume of the tissue, and the partition coefficients.

The results of the same limited optimization, now with additional parameters V_{MAX}Clara, K_MClara, and D being estimated simultaneously with the hepatic V_{MAX} and K_M, are shown in the bottom panels of Figures A-2 and A-3. The improvement in the model fits is obvious, and these results served as a motivation to include this respiratory metabolism model for analysis by the more formal Bayesian methods.

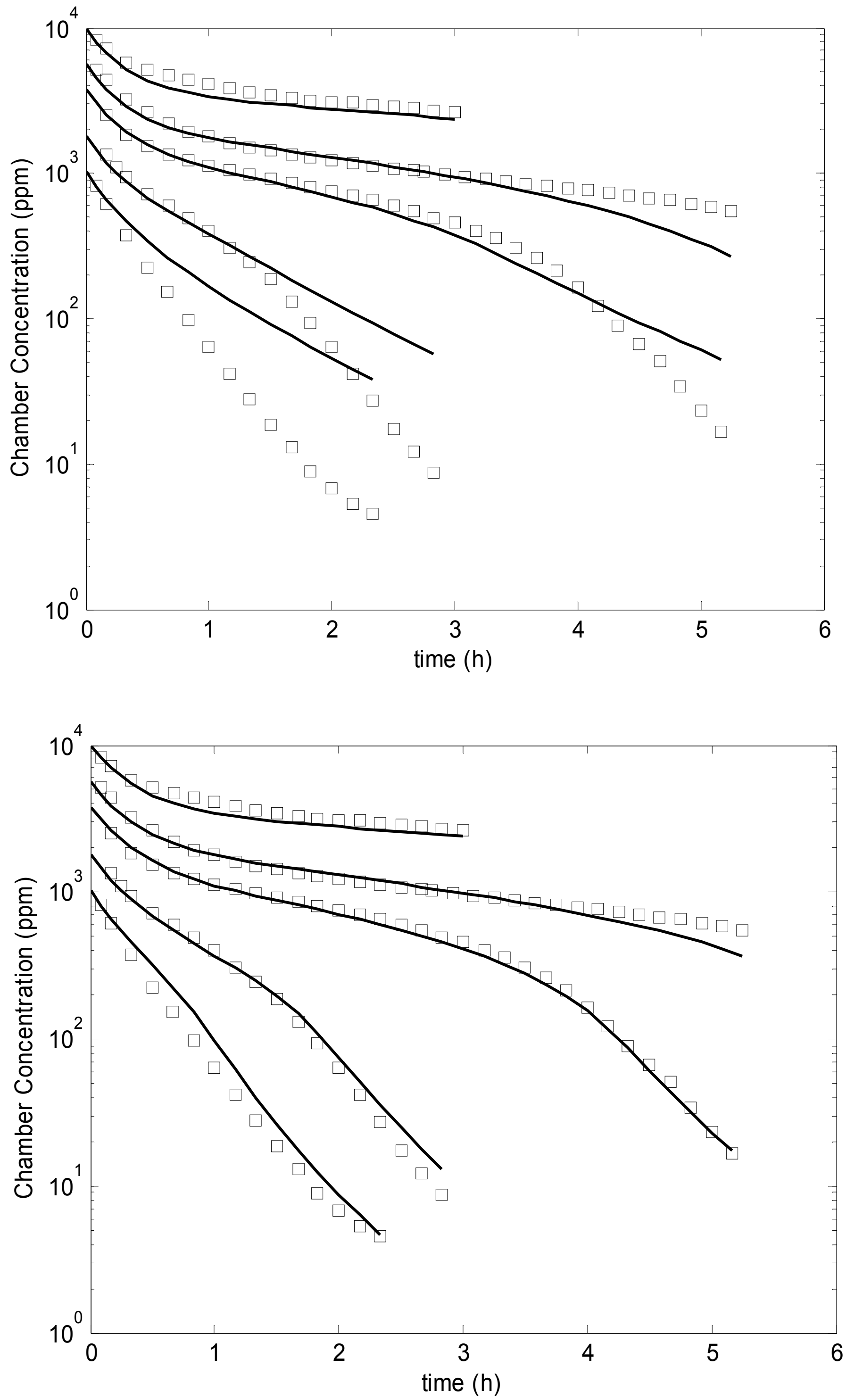

Figure A-3. Limited optimization results for male closed chamber data from Fisher et al. (1991) without (top) and with (bottom) respiratory metabolism.

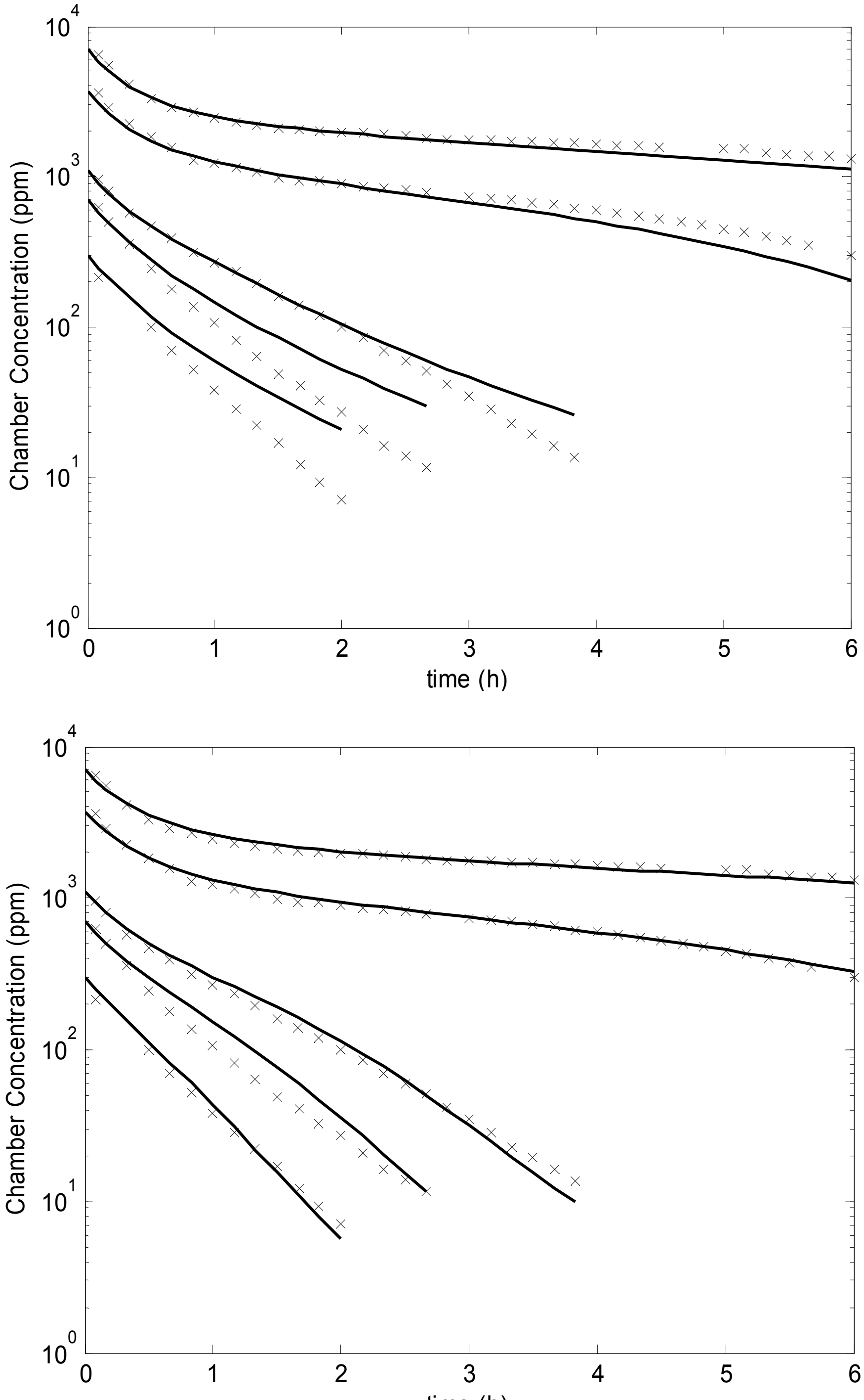

Figure A-4. Limited optimization results for female closed chamber data from Fisher et al. (1991) without (top) and with (bottom) respiratory metabolism.

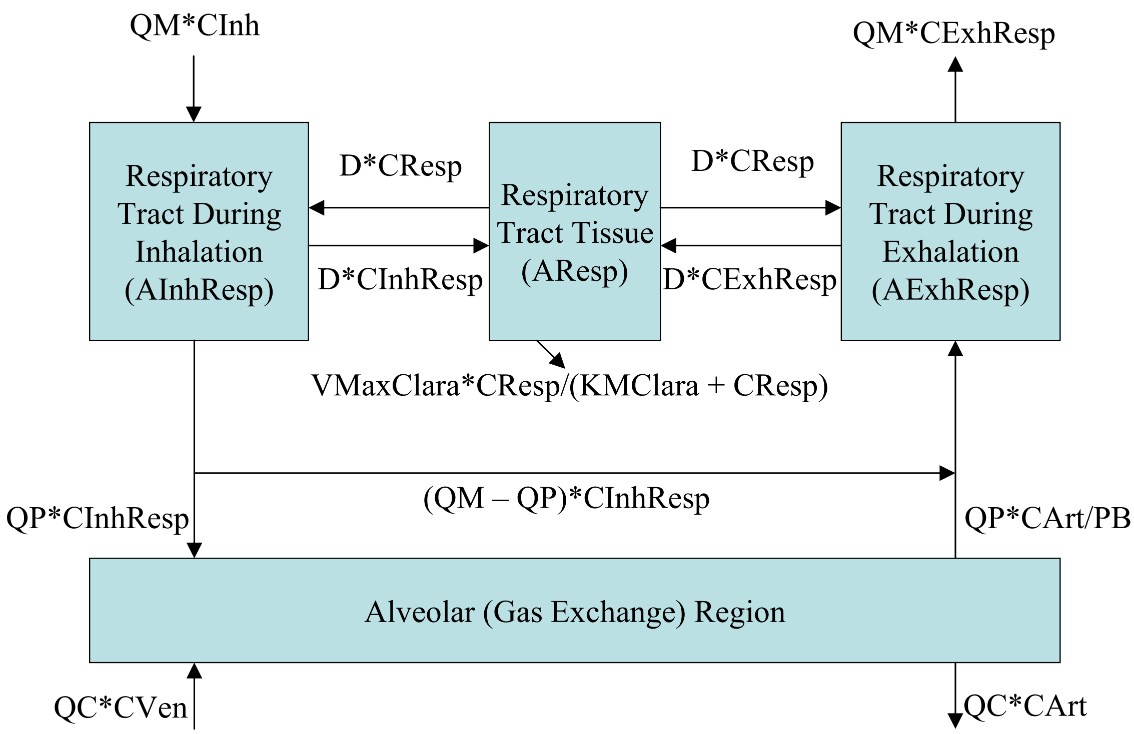

Figure A-5. Respiratory metabolism model for updated PBPK model.

A.4. DETAILS OF THE UPDATED PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR TRICHLOROETHYLENE (TCE) AND ITS METABOLITES

The structure of the updated PBPK model and the statistical population model are shown graphically in Chapter 3, with the model code shown below in Section A.6. Details as to its parameter values and their prior distributions are given below.

A.4.1. Model Parameters and Baseline Values

The multipage Table A-4 below describes all the parameters of the updated PBPK model, their baseline values (which are used as central estimates in the prior distributions for the Bayesian analysis), and any scaling relationship used in their calculation. More detailed notes are included in the comments of the model code (see Section A.6).

Table A-4. PBPK model parameters, baseline values, and scaling relationships

Model parameter	Abbreviation	Baseline value (if applicable)				Scaling (Sampled) Parameter	Additional scaling (if any)	Notes/ source
				Human				
		Mouse	Rat	Female (or both)	Male			
Body weight (kg)	**BW**	0.03	0.3	60	70			a
Flows								
Cardiac output (L/h)	**QC**	11.6	13.3	16		**lnQCC**	$BW^{3/4}$	b
Alveolar ventilation (L/h)	**QP**	2.5	1.9	0.96		**lnVPRC**	QC	c
Respiratory lumen:tissue diffusion flow rate (L/h)	**DResp**					**lnDRespC**	QP	d
Physiological blood flows to tissues								
Fat blood flow	**QFat**	0.07	0.07	0.085	0.05	**QFatC**	QC	e
Gut blood flow (portal vein)	**QGut**	0.141	0.153	0.21	0.19	**QGutC**	QC	e
Liver blood flow (hepatic artery)	**QLiv**	0.02	0.021	0.065		**QLivC**	QC	e
Slowly perfused blood flow	**QSlw**	0.217	0.336	0.17	0.22	**QSlwC**	QC	e
Kidney blood flow	**QKid**	0.091	0.141	0.17	0.19	**QKidC**	QC	e
Rapidly perfused blood flow	**QRap**							e
Fraction of blood that is plasma	**FracPlas**	0.52	0.53	0.615	0.567	**FracPlasC**		f
Physiological volumes								
Fat compartment volume (L)	**VFat**	0.07	0.07	0.317	0.199	**VFatC**	BW	g
Gut compartment volume (L)	**VGut**	0.049	0.032	0.022	0.02	**VGutC**	BW	g
Liver compartment volume (L)	**VLiv**	0.055	0.034	0.023	0.025	**VLivC**	BW	g
Rapidly perfused compartment volume (L)	**VRap**	0.1	0.088	0.093	0.088	**VRapC**	BW	g
Volume of respiratory lumen (L air)	**VRespLum**	0.004667	0.004667	0.002386		**VRespLumC**	BW	g
Effective volume for respiratory tissue (L air)	**VRespEff**	0.0007	0.0005	0.00018	0.00018	**VRespEffC**	BW x PResp x PB	g
Kidney compartment volume (L)	**VKid**	0.017	0.007	0.0046	0.0043	**VKidC**	BW	g
Blood compartment volume (L)	**VBld**	0.049	0.074	0.068	0.077	**VBldC**	BW	g
Total perfused volume (L)	**VPerf**	0.8897	0.8995	0.85778	0.8560		BW	g

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Model parameter	Abbreviation	Baseline value (if applicable)				Scaling (Sampled) Parameter	Additional scaling (if any)	Notes/ source
		Mouse	Rat	Human Female (or both)	Human Male			
Slowly perfused compartment volume (L)	**VSlw**							g
Plasma compartment volume (L)	**VPlas**							h
TCA body compartment volume (L)	**VBod**							i
TCOH/G body compartment volume (L)	**VBodTCOH**							j
TCE distribution/partitioning								
TCE blood/air partition coefficient	**PB**	15	22	9.5		**lnPBC**		k
TCE fat/blood partition coefficient	**PFat**	36	27	67		**lnPFatC**		l
TCE gut/blood partition coefficient	**PGut**	1.9	1.4	2.6		**lnPGutC**		m
TCE liver/blood partition coefficient	**PLiv**	1.7	1.5	4.1		**lnPLivC**		n
TCE rapidly perfused/blood partition coefficient	**PRap**	1.9	1.3	2.6		**lnPRapC**		o
TCE respiratory tissue:air partition coefficient	**PResp**	2.6	1	1.3		**lnPRespC**		p
TCE kidney/blood partition coefficient	**PKid**	2.1	1.3	1.6		**lnPKidC**		q
TCE slowly perfused/blood partition coefficient	**PSlw**	2.4	0.58	2.1		**lnPSlwC**		r
TCA distribution/partitioning								
TCA blood/plasma concentration ratio	**TCAPlas**	0.5	0.5	0.5		**lnPRBCPlasTCAC**	See note	s
Free TCA body/blood plasma partition coefficient	**PBodTCA**	0.88	0.88	0.52		**lnPBodTCAC**		t
Free TCA liver/blood plasma partition coefficient	**PLivTCA**	1.18	1.18	0.66		**lnPLivTCAC**		t
TCA plasma binding								
Protein/TCA dissociation constant (µmol/L)	**kDissoc**	107	275	182		**lnkDissocC**		u
Protein concentration (umole/L)	**BMax**	0.88	1.22	4.62		**lnBMaxkDC**		u

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Model parameter	Abbreviation	Baseline value (if applicable)				Scaling (Sampled) Parameter	Additional scaling (if any)	Notes/ source
		Mouse	Rat	Human: Female (or both)	Human: Male			
TCOH and TCOG distribution/partitioning								
TCOH body/blood partition coefficient	**PBodTCOH**	1.11	1.11	0.91		**lnPBodTCOHC**		v
TCOH liver/body partition coefficient	**PLivTCOH**	1.3	1.3	0.59		**lnPLivTCOHC**		v
TCOG body/blood partition coefficient	**PBodTCOG**	1.11	1.11	0.91		**lnPBodTCOGC**		w
TCOG liver/body partition coefficient	**PLivTCOG**	1.3	1.3	0.59		**lnPLivTCOGC**		w
DCVG distribution/partitioning								
DCVG effective volume of distribution	**VDCVG**					**lnPeffDCVG**	See note	x
TCE metabolism								
V_{MAX} for hepatic TCE oxidation (mg/h)	**V_{MAX}**	2,700	600	255		**lnV_{MAX}C**	VLiv	y
K_M for hepatic TCE oxidation (mg/L)	**K_M**	36	21			**lnK_MC**		y
				66		**lnClC**	See note	y
Fraction of hepatic TCE oxidation not to TCA+TCOH	**FracOther**					**lnFracOtherC**	See note	z
Fraction of hepatic TCE oxidation to TCA	**FracTCA**	0.32	0.32	0.32		**lnFracTCAC**	See note	aa
V_{MAX} for hepatic TCE GSH conjugation (mg/h)	**V_{MAX}DCVG**	300	66			**lnV_{MAX}DCVGC**	VLiv	bb
		1.53	0.25	19		**lnClDCVGC**		bb
K_M for hepatic TCE GSH conjugation (mg/L)	**K_MDCVG**			2.9		**lnK_MDCVGC**		bb
V_{MAX} for renal TCE GSH conjugation (mg/h)	**V_{MAX}KidDCVG**	60	6			**lnV_{MAX}KidDCVGC**	VKid	bb
		0.34	0.026	230		**lnClKidDCVGC**		bb
K_M for renal TCE GSH conjugation (mg/L)	**K_MKidDCVG**			2.7		**lnK_MKidDCVGC**		bb
TCE metabolism (respiratory tract)								
V_{MAX} for tracheo-bronchial TCE oxidation (mg/h)	**V_{MAX}Clara**	0.070102	0.014347	0.027273	0.025253	**lnV_{MAX}LungLivC**	V_{MAX}	cc
K_M for tracheo-bronchial TCE oxidation (mg/L air)	**K_MClara**					**lnK_MClara**		cc

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Model parameter	Abbreviation	Baseline value (if applicable)				Scaling (Sampled) Parameter	Additional scaling (if any)	Notes/ source
		Mouse	Rat	Human: Female (or both)	Human: Male			
Fraction of respiratory oxidation entering systemic circulation	**FracLungSys**					**lnFracLungSysC**	See note	dd
TCOH metabolism								
V_{MAX} for hepatic TCOH->TCA (mg/h)	**V_{MAX}TCOH**					**lnV_{MAX}TCOHC**	$BW^{3/4}$	
						lnClTCOHC	$BW^{3/4}$	
K_M for hepatic TCOH->TCA (mg/L)	**K_MTCOH**					**lnK_MTCOH**		
V_{MAX} for hepatic TCOH->TCOG (mg/h)	**V_{MAX}Gluc**					**lnV_{MAX}GlucC**	$BW^{3/4}$	
						lnClGlucC	$BW^{3/4}$	
K_M for hepatic TCOH->TCOG (mg/L)	**K_MGluc**					**lnK_MGluc**		
Rate constant for hepatic TCOH->other (/h)	**kMetTCOH**					**lnkMetTCOHC**	$BW^{-1/4}$	
TCA metabolism/clearance								
Rate constant for TCA plasma->urine (/h)	**kUrnTCA**	0.6	0.522	0.108		**lnkUrnTCAC**	$VPlas^{-1}$	ee
Rate constant for hepatic TCA->other (/h)	**kMetTCA**					**lnkMetTCAC**	$BW^{-1/4}$	
TCOG metabolism/clearance								
Rate constant for TCOG liver->bile (/h)	**kBile**					**lnkBileC**	$BW^{-1/4}$	
Lumped rate constant for TCOG bile->TCOH liver (/h)	**kEHR**					**lnkEHRC**	$BW^{-1/4}$	
Rate constant for TCOG->urine (/h)	**kUrnTCOG**	0.6	0.522	0.108		**lnkUrnTCOGC**	$VBld^{-1}$	ee
DCVG metabolism								
Rate constant for hepatic DCVG->DCVC (/h)	**kDCVG**					**lnkDCVGC**	$BW^{-1/4}$	ff

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

Model parameter	Abbreviation	Baseline value (if applicable)				Scaling (Sampled) Parameter	Additional scaling (if any)	Notes/ source
		Mouse	Rat	Human				
				Female (or both)	Male			
DCVC metabolism/clearance								
Lumped rate constant for DCVC->Urinary NAcDCVC (/h)	**kNAT**					**lnkNATC**	$BW^{-1/4}$	gg
Rate constant for DCVC bioactivation (/h)	**kKidBioact**					**lnkKidBioactC**	$BW^{-1/4}$	gg
Oral uptake/transfer coefficients								
TCE Stomach-duodenum transfer coefficient (/h)	**kTSD**					**lnkTSD**		hh
TCE stomach absorption coefficient (/h)	**kAS**					**lnkAS**		hh
TCE duodenum absorption coefficient (/h)	**kAD**					**lnkAD**		hh
TCA stomach absorption coefficient (/h)	**kASTCA**					**lnkASTCA**		hh
TCOH stomach absorption coefficient (/h)	**kASTCOH**					**lnkASTCOH**		hh

Explanatory note. Unless otherwise noted, the model parameter is obtained by multiplying (1) the "baseline value" (equals 1 if not specified) times (2) the scaling parameter [or for those beginning with "ln," which are natural-log transformed, exp(lnXX)] times (3) any additional scaling as noted in the second to last column. Unless otherwise noted, all log-transformed scaling parameters have baseline value of 0 [i.e., exp(lnXX) has baseline value of 1] and all other scaling parameters have baseline parameters of 1.

[a]Use measured value if available.
[b]If QP is measured, then scale by QP using VPR. Baseline values are from Brown et al. (1997) (mouse and rat) and ICRP (International Commission on Radiological Protection) Publication 89 (2003) (human).
[c]Use measured QP, if available; otherwise scale by QC using alveolar VPR. Baseline values are from Brown et al. (1997) (mouse and rat) and ICRP Publication 89 (2003) (human).
[d]Scaling parameter is relative to alveolar ventilation rate.
[e]Fat represents adipose tissue only. Gut is the gastro-intestinal tract, pancreas, and spleen (all drain to the portal vein). Slowly perfused tissue is the muscle and skin. Rapidly perfused tissue is the rest of the organs, plus the bone marrow and lymph nodes, the blood flow for which is calculated as the difference between QC and the sum of the other blood flows. Baseline values are from Brown et al. (1997) (mouse and rat) and ICRP Publication 89 (2003) (human).
[f]This is equal to 1 minus the hematocrit (measured value used if available). Baseline values from control animals in Hejtmancik et al. (2002) (mouse and rat) and ICRP Publication 89 (2003) (human).

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

[g]Fat represents adipose tissue only, and the measured value is used, if available. Gut is the gastro-intestinal tract, pancreas, and spleen (all drain to the portal vein). Rapidly perfused tissue is the rest of the organs, plus the bone marrow and lymph nodes, minus the tracheobronchial region. The respiratory tissue volume is tracheobronchial region, with an effective air volume given by multiplying by its tissue:air partition coefficient (= tissue:blood times blood:air). The slowly perfused tissue is the muscle and skin. This leaves a small (10−15% of body weight [BW]) unperfused volume that consists mostly of bone (minus marrow) and the gastro-intestinal tract contents. Baseline values are from Brown et al. (1997) (mouse and rat) and ICRP Publication 89 (2003) (human), except for volumes of the respiratory lumen, which are from Sarangapani et al. (2003).

[h]Derived from blood volume using FracPlas.

[i]Sum of all compartments except the blood and liver.

[j]Sum of all compartments except the liver.

[k]Mouse value is from pooling Abbas and Fisher (1997) and Fisher et al. (1991). Rat value is from pooling Sato et al. (1977), Gargas et al. (1989), Barton et al. (1995), Simmons et al. (2002), Koizumi (1989), and Fisher et al. (1989). Human value is from pooling Sato and Nakajima (1979), Sato et al. (1977), Gargas et al. (1989), Fiserova-Bergerova et al. (1984), Fisher et al. (1998), and Koizumi (1989).

[l]Mouse value is from Abbas and Fisher (1997). Rat value is from pooling Barton et al. (1995), Sato et al. (1977), and Fisher et al. (1989). Human value is from pooling Fiserova-Bergerova et al. (1984), Fisher et al. (1998), and Sato et al. (1977).

[m]Value is the geometric mean of liver and kidney (relatively high uncertainty) values.

[n]Mouse value is from Fisher et al. (1991). Rat value is from pooling Barton et al. (1995), Sato et al. (1977), and Fisher et al. (1989). Human value is from pooling Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).

[o]Mouse value is geometric mean of liver and kidney values. Rat value is the brain value from Sato et al. (1977). Human value is the brain value from Fiserova-Bergerova et al. (1984).

[p]Mouse value is the lung value from Abbas and Fisher (1997). Rat value is the lung value from Sato et al. (1977). Human value is from pooling lung values from Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).

[q]Mouse value is from Abbas and Fisher (1997). Rat value is from pooling Barton et al. (1995) and Sato et al. (1977). Human value is from pooling Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).

[r]Mouse value is the muscle value from Abbas and Fisher (1997). Rat value is the muscle value from pooling Barton et al. (1995), Sato et al. (1977), and Fisher et al. (1989). Human value is the muscle value from pooling Fiserova-Bergerova et al. (1984) and Fisher et al. (1998).

[s]Scaling parameter is the effective partition coefficient between red blood cells and plasma. Thus, the TCA blood-plasma concentration ratio depends on the plasma fraction. Baseline value is based on the blood-plasma concentration ratio of 0.76 in rats (Schultz et al., 1999).

[t]*In vitro* partition coefficients were determined at high concentration, when plasma binding is saturated, so should reflect the free blood:tissue partition coefficient. To get the plasma partition coefficient, the partition coefficient is multiplied by the blood:plasma concentration ratio (TCAPlas). *In vitro* values were from Abbas and Fisher (1997) in the mouse (used for both mouse and rat) and from Fisher et al. (1998). Body values based on measurements in muscle.

[u]Values are based on the geometric mean of estimates based on data from Lumpkin et al. (2003), Schultz et al. (1999), Templin et al. (1993, 1995), and Yu et al. (2000). Scaling parameter for B_{MAX} is actually the ratio of B_{MAX}/kD, which determines the binding at low concentrations.

[v]Data are from Abbas and Fisher (1997) in the mouse (used for the mouse and rat) and Fisher et al. (1998) (human).

[w]Used *in vitro* measurements in TCOH as a proxy, but higher uncertainty is noted.

[x]The scaling parameter (only used in the human model) is the effective partition coefficient for the “body” (nonblood) compartment, so that the distribution volume VDCVG is given by VBld + exp(lnPeffDCVG) × (VBod + VLiv).

Table A-4. PBPK model parameters, baseline values, and scaling relationships (continued)

[y]Baseline values have the following units: for V_{Max}, mg/hour/kg liver; for K_M, mg/L blood; and for clearance (Cl), L/hour/kg liver (in humans, K_M is calculated from $K_M = V_{Max}/(\exp(\ln ClC) \times Vliv)$. Values are based on *in vitro* (microsomal and hepatocellular preparations) from Elfarra et al. (1998), Lipscomb et al. (1997, 1998a, b). Scaling from *in vitro* data based on 32 mg microsomal protein/g liver and 99 × 106 hepatocytes/g liver (Barter et al., 2007). Scaling of K_M from microsomes were based on two methods: (1) assuming microsomal concentrations equal to liver tissue concentrations and (2) using the measured microsome:air partition coefficient and a central estimate of the blood:air partition coefficient. For K_M from human hepatocyte preparations, the measured hepatocyte:air partition coefficient and a central estimate of the blood:air partition coefficient was used.

[z]Scaling parameter is ratio of "DCA" to "non-DCA" oxidative pathway (where DCA is a proxy for oxidative metabolism not producing TCA or TCOH). Fraction of "other" oxidation is exp(lnFracOtherC)/(1 + exp[lnFracOtherC]).

[aa]Scaling parameter is ratio of TCA to TCOH pathways. Baseline value based on geometric mean of Lipscomb et al. (1998b) using fresh hepatocytes and Bronley-DeLancey et al. (2006) using cryogenically-preserved hepatocytes. Fraction of oxidation to TCA is (1 − FracOther) × exp(lnFracTCAC)/(1 + exp[lnFracTCAC]).

[bb]Baseline values are based on *in vitro* data. In the mouse and rat, the only *in vitro* data are at 1 or 2 mM (Lash et al., 1995, 1998). In most cases, rates at 2 mM were increased over the same sex/species at 1 mM, indicating V_{Max} has not yet been reached. These data therefore put lower bounds on both V_{Max} (in units of mg/hour/kg tissue) and clearance (in units of L/hour/kg tissue), so those are the scaling parameters used, with those bounds used as baseline values. For humans, data from Lash et al. (1999a) in the liver (hepatocytes) and the kidney (cytosol) and Green et al. (1997) (liver cytosol) was used to estimate the clearance in units of L/hour/kg tissue and K_M in units of mg/L in blood.

[cc]Scaling parameter is the ratio of the lung to liver V_{Max} (each in units of mg/hour), with baseline values based on microsomal preparations (mg/hour/mg protein) assayed at ~1 mM (Green et al., 1997), further adjusted by the ratio of lung to liver tissue masses (Brown et al., 1997; ICRP Publication 89 [2003]).

[dd]Scaling parameter is the ratio of respiratory oxidation entering systemic circulation (translocated to the liver) to that locally cleared in the lung. Fraction of respiratory oxidation entering systemic circulation is exp(lnFracLungSysC)/(1 + exp[lnFracLungSysC]).

[ee]Baseline parameters for urinary clearance (L/hour) were based on glomular filtration rate per unit body weight (L/hour/kg BW) from Lin (1995), multiplied by the body weights cited in the study. For TCA, these were scaled by plasma volume to obtain the rate constant (/hour), since the model clears TCA from plasma. For TCOG, these were scaled by the effective distribution volume of the body (VBodTCOH × PBodTCOG) to obtain the rate constant (/hour), since the model clears TCOG from the body compartment.

[ff]Human model only.

[gg]Rat and human models only.

[hh]Baseline value for oral absorption scaling parameter are as follows: kTSD and kAS, 1.4/hour, based on human stomach half time of 0.5 hour; kAD, kASTCA, and kASTCOH, 0.75/hour, based on human small intestine transit time of 4 hours (ICRP Publication 89, 2003). These are noted to have very high uncertainty.

DCVG = S-dichlorovinyl glutathione.

A.4.2. Statistical Distributions for Parameter Uncertainty and Variability

A.4.2.1. *Initial Prior Uncertainty in Population Mean Parameters*

The following multipage Table A-5 describes the initial prior distributions for the population mean of the PBPK model parameters. For selected parameters, rat prior distributions were subsequently updated using the mouse posterior distributions, and human prior distributions were then updated using mouse and rat posterior distributions (see Section A.4.2.2).

A.4.2.2. *Interspecies Scaling to Update Selected Prior Distributions in the Rat and Human*

As shown in Table A-5, for several parameters, there is little or no *in vitro* or other prior information available to develop informative prior distributions, so many parameters had lognormal or log-uniform priors that spanned a wide range. Initially, the PBPK model for each species was run with the initial prior distributions in Table A-5, but, in the time available for analysis (up to about 100,000 iterations), only for the mouse did all these parameters achieve adequate convergence. Additional preliminary runs indicated replacing the log-uniform priors with lognormal priors and/or requiring more consistency between species could lead to adequate convergence. However, an objective method of "centering" the lognormal distributions that did not rely on the *in vivo* data (e.g., via visual fitting or limited optimization) being calibrated against was necessary in order to minimize potential bias.

Therefore, the approach taken was to consider three species sequentially, from mouse to rat to human, and to use a model for interspecies scaling to update the prior distributions across species (the original prior distributions define the prior bounds). This sequence was chosen because the models are essentially "nested" in this order—the rat model adds to the mouse model the "downstream" GSH conjugation pathways, and the human model adds to the rat model the intermediary S-dichlorovinyl glutathione (DCVG) compartment. Therefore, for those parameters with little or no independent data *only*, the mouse posteriors were used to update the rat priors, and both the mouse and rat posteriors were used to update the human priors. A list of the parameters for which this scaling was used to update prior distributions is contained in Table A-6, with the updated prior distributions. The correspondence between the "scaling parameters" and the physical parameters generally follows standard practice, and were explicitly described in Table A-4. For instance, V_{MAX} and clearance rates are scaled by body weight to the ¾ power, whereas K_M values are assumed to have no scaling, and rate constants (inverse time units) are scaled by body weight to the –¼ power.

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	
Flows										
lnQCC	TruncNormal	0.2	4	TruncNormal	0.14	4	TruncNormal	0.2	4	[a]
lnVPRC	TruncNormal	0.2	4	TruncNormal	0.3	4	TruncNormal	0.2	4	[a]
lnDRespC	Uniform	-11.513	2.303	Uniform	-11.513	2.303	Uniform	-11.513	2.303	[b]
Physiological blood flows to tissues										
QFatC	TruncNormal	0.46	2	TruncNormal	0.46	2	TruncNormal	0.46	2	[a]
QGutC	TruncNormal	0.17	2	TruncNormal	0.17	2	TruncNormal	0.18	2	[a]
QLivC	TruncNormal	0.17	2	TruncNormal	0.17	2	TruncNormal	0.45	2	[a]
QSlwC	TruncNormal	0.29	2	TruncNormal	0.3	2	TruncNormal	0.32	2	[a]
QKidC	TruncNormal	0.32	2	TruncNormal	0.13	2	TruncNormal	0.12	2	[a]
FracPlasC	TruncNormal	0.2	3	TruncNormal	0.2	3	TruncNormal	0.05	3	[c]
Physiological volumes										
VFatC	TruncNormal	0.45	2	TruncNormal	0.45	2	TruncNormal	0.45	2	[a]
VGutC	TruncNormal	0.13	2	TruncNormal	0.13	2	TruncNormal	0.08	2	[a]
VLivC	TruncNormal	0.24	2	TruncNormal	0.18	2	TruncNormal	0.23	2	[a]
VRapC	TruncNormal	0.1	2	TruncNormal	0.12	2	TruncNormal	0.08	2	[a]
VRespLumC	TruncNormal	0.11	2	TruncNormal	0.18	2	TruncNormal	0.2	2	[a]
VRespEffC	TruncNormal	0.11	2	TruncNormal	0.18	2	TruncNormal	0.2	2	[a]
VKidC	TruncNormal	0.1	2	TruncNormal	0.15	2	TruncNormal	0.17	2	[a]
VBldC	TruncNormal	0.12	2	TruncNormal	0.12	2	TruncNormal	0.12	2	[a]

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	
TCE distribution/partitioning										
lnPBC	TruncNormal	0.25	3	TruncNormal	0.25	3	TruncNormal	0.2	3	[d]
lnPFatC	TruncNormal	0.3	3	TruncNormal	0.3	3	TruncNormal	0.2	3	
lnPGutC	TruncNormal	0.4	3	TruncNormal	0.4	3	TruncNormal	0.4	3	
lnPLivC	TruncNormal	0.4	3	TruncNormal	0.15	3	TruncNormal	0.4	3	
lnPRapC	TruncNormal	0.4	3	TruncNormal	0.4	3	TruncNormal	0.4	3	
lnPRespC	TruncNormal	0.4	3	TruncNormal	0.4	3	TruncNormal	0.4	3	
lnPKidC	TruncNormal	0.4	3	TruncNormal	0.3	3	TruncNormal	0.2	3	
lnPSlwC	TruncNormal	0.4	3	TruncNormal	0.3	3	TruncNormal	0.3	3	
TCA distribution/partitioning										
lnPRBCPlasTCAC	Uniform	-4.605	4.605	TruncNormal	0.336	3	Uniform	-4.605	4.605	[e]
lnPBodTCAC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	[f]
lnPLivTCAC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	
TCA plasma binding										
lnkDissocC	TruncNormal	1.191	3	TruncNormal	0.61	3	TruncNormal	0.06	3	[g]
lnBMaxkDC	TruncNormal	0.495	3	TruncNormal	0.47	3	TruncNormal	0.182	3	
TCOH and TCOG distribution/partitioning										
lnPBodTCOHC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	
lnPLivTCOHC	TruncNormal	0.336	3	TruncNormal	0.693	3	TruncNormal	0.336	3	
lnPBodTCOGC	Uniform	-4.605	4.605	Uniform	-4.605	4.605	Uniform	-4.605	4.605	
lnPLivTCOGC	Uniform	-4.605	4.605	Uniform	-4.605	4.605	Uniform	-4.605	4.605	

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	
DCVG distribution/partitioning										
lnPeffDCVG	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	h
TCE Metabolism										
lnV_{MAX}C	TruncNormal	0.693	3	TruncNormal	0.693	3	TruncNormal	0.693	3	i
lnK_MC	TruncNormal	1.386	3	TruncNormal	1.386	3				i
lnClC							TruncNormal	1.386	3	i
lnFracOtherC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	h
lnFracTCAC	TruncNormal	1.163	3	TruncNormal	1.163	3	TruncNormal	1.163	3	j
lnV_{MAX}DCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21				k
lnClDCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21	TruncNormal	4.605	3	k
lnK_MDCVGC							TruncNormal	1.386	3	k
lnV_{MAX}KidDCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21				k
lnClKidDCVGC	Uniform	-4.605	9.21	Uniform	-4.605	9.21	TruncNormal	4.605	3	k
lnK_MKidDCVGC							TruncNormal	1.386	3	k
lnV_{MAX}LungLivC	TruncNormal	1.099	3	TruncNormal	1.099	3	TruncNormal	1.099	3	l
lnK_MClara	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	h
lnFracLungSysC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	h
TCOH metabolism										
lnV_{MAX}TCOHC	Uniform	-9.21	9.21	Uniform	-9.21	9.21				h
lnClTCOHC							Uniform	-11.513	6.908	
lnK_MTCOH	Uniform	-9.21	9.21	Uniform	-9.21	9.21	Uniform	-9.21	9.21	
lnV_{MAX}GlucC	Uniform	-9.21	9.21	Uniform	-9.21	9.21				
lnClGlucC							Uniform	-9.21	4.605	
lnK_MGluc	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	h
lnkMetTCOHC	Uniform	-11.513	6.908	Uniform	-11.513	6.908	Uniform	-11.513	6.908	

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse			Rat			Human			Notes/ Source
	Distribution[a]	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	Distribution	*SD or Min*	*Truncation (±nxSD) or Max*	
TCA metabolism/clearance										
lnkUrnTCAC	Uniform	-4.605	4.605	Uniform	-4.605	4.605	Uniform	-4.605	4.605	[h]
lnkMetTCAC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	
TCOG metabolism/clearance										
lnkBileC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	[h]
lnkEHRC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	
lnkUrnTCOGC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	
DCVG metabolism										
lnFracKidDCVCC	Uniform	-6.908	6.908	Uniform	-6.908	6.908	Uniform	-6.908	6.908	[h]
lnkDCVGC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	
DCVC metabolism/clearance										
lnkNATC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	[h]
lnkKidBioactC	Uniform	-9.21	4.605	Uniform	-9.21	4.605	Uniform	-9.21	4.605	
Oral uptake/transfer coefficients										
lnkTSD	Uniform	-4.269	4.942	Uniform	-4.269	4.942	Uniform	-4.269	4.942	[h]
lnkAS	Uniform	-6.571	7.244	Uniform	-6.571	7.244	Uniform	-6.571	7.244	
lnkTD	Uniform	-4.605	0	Uniform	-4.605	0	Uniform	-4.605	0	
lnkAD	Uniform	-7.195	6.62	Uniform	-7.195	6.62	Uniform	-7.195	6.62	
lnkASTCA	Uniform	-7.195	6.62	Uniform	-7.195	6.62	Uniform	-7.195	6.62	[h]
lnkASTCOH	Uniform	-7.195	6.62	Uniform	-7.195	6.62	Uniform	-7.195	6.62	

Explanatory note. All population mean parameters have either truncated normal (TruncNormal) or uniform distributions. For those with TruncNormal distributions, the mean for the population mean is 0 for natural-log transformed parameters (parameter name starting with "ln") and 1 for untransformed parameters, with the truncation at the specified number (*n*) of standard deviations (SD). All uniformly distributed parameters are natural-log transformed, so their untransformed minimum and maximum are exp(Min) and exp(Max), respectively.

Table A-5. Uncertainty distributions for the population mean of the PBPK model parameters (continued)

[a]Uncertainty based on CV or range of values in Brown et al. (1997) (mouse and rat) and a comparison of values from ICRP Publication 89 (2003), Brown et al. (1997), and Price et al. (2003) (human).

[b]Noninformative prior distribution intended to span a wide range of possibilities because no independent data are available on these parameters. These priors for the rat and human were subsequently updated (see Section A.4.2.2).

[c]Because of potential strain differences, uncertainty in mice and rat assumed to be 20%. In humans, Price et al. (2003) reported variability of about 5%, and this is also used for the uncertainty in the mean.

[d]For partition coefficients, it is not clear whether interstudy variability is due to interindividual or assay variability, so uncertainty in the mean is based on interstudy variability among *in vitro* measurements. For single measurements, uncertainty SD of 0.3 was used for fat (mouse) and 0.4 for other tissues was used. In addition, where measurements were from a surrogate tissue (e.g., gut was based on liver and kidney), an uncertainty SD 0.4 was used.

[e]Single *in vitro* data point available in rats, so a geometric standard deviation (GSD) of 1.4 was used. In mice and humans, where no *in vitro* data was available, a noninformative prior was used.

[f]Single *in vitro* data points available in mice and humans, so a GSD of 1.4 was used. In rats, where the mouse data was used as a surrogate, a GSD of 2.0 was used, based on the difference between mice and rats *in vitro*.

[g]GSD for uncertainty based on different estimates from different *in vitro* studies.

[h]Noninformative prior distribution.

[i]Assume 2-fold uncertainty GSD in V_{Max}, based on observed variability and uncertainties of *in vitro*-to-*in vivo* scaling. For K_M and ClC, the uncertainty is assumed to be 4-fold, due to the different methods for scaling of concentrations from TCE in the *in vitro* medium to TCE in blood.

[j]Uncertainty GSD of 3.2-fold reflects difference between *in vitro* measurements from Lipscomb et al. (1998b) and Bronley-DeLancey et al. (2006).

[k]In mice and rats, the baseline values are notional lower-limits on V_{Max} and clearance, however, the lower bound of the prior distribution is set to 100-fold less because of uncertainty in *in vitro-in vivo* extrapolation, and because Green et al. (1997) reported values 100-fold smaller than Lash et al. (1995, 1998). In humans, the uncertainty GSD in clearance is assumed to be 100-fold, due to the difference between Lash et al. (1998) and Green et al. (1997). For K_M, the uncertainty GSD of 4-fold is based on differences between concentrations in cells and cytosol.

[l]Uncertainty GSD of 3-fold was assumed due to possible differences in microsomal protein content, the fact that measurements were at a single concentration, and the fact that the human baseline values was based on the limit of detection.

DCVG = S-dichlorovinyl glutathione, SD = standard deviation.

Table A-6. Updated prior distributions for selected parameters in the rat and human

Scaling parameter	Initial prior bounds		Updated rat prior		Updated human prior	
	exp(min)	exp(max)	exp(μ)	exp(σ)	exp(μ)	exp(σ)
lnDRespC	1.00E-05	1.00E+01	1.22	5.21	1.84	4.18
lnPBodTCOGC	1.00E-02	1.00E+02	0.42	5.47	0.81	5.10
lnPLivTCOGC	1.00E-02	1.00E+02	1.01	5.31	2.92	4.31
lnFracOtherC	1.00E-03	1.00E+03	0.02	6.82	0.14	4.76
lnV$_{MAX}$DCVGC	1.00E-02	1.00E+04	2.61	42.52		
lnClDCVGC	1.00E-02	1.00E+04	0.36	15.03		
lnV$_{MAX}$KidDCVGC	1.00E-02	1.00E+04	2.56	22.65		
lnClKidDCVGC	1.00E-02	1.00E+04	1.22	15.03		
lnV$_{MAX}$LungLivC	3.70E-02	2.70E+01	2.77	6.17	2.80	4.71
lnK$_{M}$Clara	1.00E-03	1.00E+03	0.01	6.69	0.02	4.85
lnFracLungSysC	1.00E-03	1.00E+03	4.39	11.13	3.10	8.08
lnV$_{MAX}$TCOHC	1.00E-04	1.00E+04	1.65	5.42		
lnClTCOHC	1.00E-05	1.00E+03			0.37	4.44
lnK$_{M}$TCOH	1.00E-04	1.00E+04	0.93	5.64	4.81	4.53
lnV$_{MAX}$GlucC	1.00E-04	1.00E+04	69.41	5.58		
lnClGlucC	1.00E-04	1.00E+02			3.39	4.35
lnK$_{M}$Gluc	1.00E-03	1.00E+03	30.57	6.11	11.13	4.57
lnkMetTCOHC	1.00E-05	1.00E+03	3.35	5.87	2.39	4.62
lnkUrnTCAC	1.00E-02	1.00E+02	0.11	5.42	0.09	4.22
lnkMetTCAC	1.00E-04	1.00E+02	0.61	5.37	0.45	4.26
lnkBileC	1.00E-04	1.00E+02	1.01	5.70	3.39	4.44
lnkEHRC	1.00E-04	1.00E+02	0.01	6.62	0.22	4.71
lnkUrnTCOGC	1.00E-03	1.00E+03	8.58	6.05	16.12	4.81
lnkNATC	1.00E-04	1.00E+02			0.00	6.11
lnkKidBioactC	1.00E-04	1.00E+02			0.01	6.49

Notes: updated rat prior is based on the mouse posterior; and the updated human priors are based on combining the mouse and rat posteriors, except in the case of lnkNATC and lnkKidBioactC, which are unidentified in the mouse model. Columns labeled exp(min) and exp(max) are the exponentiated prior bounds; columns labeled exp(μ) and exp(σ) are the exponentiated mean and standard deviation of the updated prior distributions, which are normal distributions truncated at the prior bounds.

The scaling model is given explicitly as follows. If θ_i are the "scaling" parameters (usually also natural-log-transformed) that are actually estimated, and A is the "universal" (species-independent) parameter, then $\theta_i = A + \varepsilon_I$, where ε_i is the species-specific "departure" from the scaling relationship, assumed to be normally distributed with variance σ_ε^2. This "scatter" in the interspecies scaling relationship is assumed to have a standard deviation of

1.15 = ln(3.16), so that the un-logarithmically transformed 95% confidence interval spans about 100-fold (i.e., exp(2σ) = 10). This implies that 95% of the time, the species-specific scaling parameter is assumed be within 10-fold higher or lower than the "species-independent" value. However, the prior bounds, which generally span a wider range, are maintained so that if the data strongly imply an extreme species-specific value, it can be accommodated.

Therefore, the mouse model gives an initial estimate of "A," which is used to update the prior distribution for $\theta_r = A + \varepsilon_r$ in the rat (alternatively, since there is only one species at this stage, one could think of this as estimating the rat parameter using the mouse parameter, but with a cross-species variance is twice the allometric scatter variance). The rat and mouse together then give a "better" estimate of A, which is used to update the prior distribution for $\theta_h = A + \varepsilon_h$ in the human, with the assumed distribution for ε_h. This approach is implemented by approximating the posterior distributions by normal distributions, deriving heuristic "data" for the specific-specific parameters, and then using these pseudo-data to derive updated prior distributions for the other species parameters. Specifically, the procedure is as follows:

1. Run the mouse model.
2. Use the mouse posterior to derive the mouse "pseudo-data" D_m (equal to the posterior mean) and its uncertainty σ_m^2 (equal to the posterior variance).
3. Use the D_m as the prior mean for the rat. The prior variance for the rat is $2\sigma_\varepsilon^2 + \sigma_m^2$, which accounts for two components of species-specific departure from "species-independence" (one each for mouse and rat), and the mouse posterior uncertainty.
4. Match the rat posterior mean and variance to the values derived from the normal approximation (posterior mean = $\{D_m/(2\sigma_\varepsilon^2 + \sigma_m^2) + D_r/\sigma_r^2\}/\{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\}$; posterior variance = $\{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\}^{-1}$), and solve for the rat "data" D_r and its uncertainty σ_r^2.
5. Use, σ_m^2, and σ_r^2 to derive the updated prior mean and variance for the human model. For the mean (=$\{D_m/(\sigma_\varepsilon^2 + \sigma_m^2) + D_r/(\sigma_\varepsilon^2 + \sigma_r^2)\}/\{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}$), it is the weighted average of the mouse and rat, with each weight including both posterior uncertainty and departure from "species-independence." For the variance (=$\{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}^{-1} + \sigma_\varepsilon^2$), it is the variance in the weighted average of the mouse and rat plus an additional component of species-specific departure from "species-independence."

Formally, then, the probability of θ_i given A can be written as

$$P(\theta_i \mid A) = \varphi(\theta_i - A, \sigma_\varepsilon^2) \qquad \text{(Eq. A-5)}$$

where $\varphi(x, \sigma^2)$ is the normal density centered on 0 with variance σ^2. Let D_i be a heuristic "datum" for species i, so the likelihood given θ_i is adequately approximated by

$$P(D_i \mid \theta_i) = \varphi(D_i - \theta_i, \sigma_i^2) \qquad \text{(Eq. A-6)}$$

Therefore, considering A to have a uniform prior distribution, then running the mouse model gives a posterior of the form

$$P(A, \theta_m \mid D_m) \propto P(A)\, P(\theta_m \mid A)\, P(D_m \mid \theta_m) \propto \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2) \qquad \text{(Eq. A-7)}$$

From the MCMC posterior, the values of D_m and σ_m^2 are simply the mean and variance of the scaled parameter θ_m.

Now, adding the rat data gives

$$P(A, \theta_m, \theta_r \mid D_m, D_r) \propto P(A)\, \mathrm{P}(\theta_m \mid A)\, P(D_m \mid \theta_m)\, P(\theta_r \mid A)\, P(D_r \mid \theta_r) \qquad \text{(Eq. A-8)}$$
$$\propto \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, \varphi(D_r - \theta_r, \sigma_r^2) \qquad \text{(Eq. A-9)}$$

D_r and σ_r^2 can be derived by marginalizing first over θ_m and then over A:

$$\int P(A, \theta_m, \theta_r \mid D_m, D_r)\, d\theta_m\, dA$$
$$\propto \left[\int P(A) \left\{\int P(\theta_m \mid A)\, P(D_m \mid \theta_m)\, d\theta_m\right\} P(\theta_r \mid A)\, dA\right] P(D_r \mid \theta_r) \qquad \text{(Eq. A-10)}$$
$$= \left[\int P(A)\, P(D_m \mid A)\, \mathrm{P}(\theta_r \mid A)\, dA\right] P(D_r \mid \theta_r) \qquad \text{(Eq. A-11)}$$
$$\propto \left[\int P(A \mid D_m)\, P(\theta_r \mid A)\, dA\right] P(D_r \mid \theta_r) \qquad \text{(Eq. A-12)}$$
$$= \mathrm{P}(\theta_r \mid D_m)\, \mathrm{P}(D_r \mid \theta_r) \qquad \text{(Eq. A-13)}$$

So $P(\theta_r \mid D_m)$ can be identified as the prior for θ_r based on the mouse data, and $P(D_r \mid \theta_r)$ as the rat-specific likelihood. The updated prior for the rats is then

$$P(\theta_r \mid D_m) \propto \int \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - \mathrm{A}, \sigma_\varepsilon^2)\, d\theta_m\, dA \qquad \text{(Eq. A-14)}$$
$$= \int \varphi(D_m - A, \sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, dA \qquad \text{(Eq. A-15)}$$
$$= \varphi(D_m - \theta_r, 2\sigma_\varepsilon^2 + \sigma_m^2) \qquad \text{(Eq. A-16)}$$

Therefore, for the "mouse-based" prior, use the mean D_m from the mouse, and then the variance from the mouse σ_m^2 plus twice the "allometric scatter" variance σ_ε^2.

The rat "data" and variance, assuming conditional independence of the rat and mouse "pseudo-data," is thus

$$P(\theta_r \mid D_m, D_r) \propto P(\theta_r \mid D_m)\, P(D_r \mid \theta_r) \quad \text{(Eq. A-17)}$$
$$\propto \varphi(D_m - \theta_r, 2\sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(D_r - \theta_r, \sigma_r^2) \quad \text{(Eq. A-18)}$$

This distribution is also normal with

$$E(\theta_r) = \{D_m/(2\sigma_\varepsilon^2 + \sigma_m^2) + D_r/\sigma_r^2\}/\ \{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\} = \text{weighted mean of } D_r \quad \text{(Eq. A-19)}$$
$$\mathrm{VAR}(\theta_r) = \{1/(2\sigma_\varepsilon^2 + \sigma_m^2) + 1/\sigma_r^2\}^{-1} = \text{harmonic mean of variances} \quad \text{(Eq. A-20)}$$

Thus, using the mean and variance of the posterior distribution from the MCMC analysis, D_r and σ_r^2 can be derived.

Now, D_m, σ_m^2, D_r, and σ_r^2 are known, so the analogous "mouse+rat" based prior used in the human model can be derived. As with the rat prior, the human prior is based on a normal approximation of the posterior for A, and then incorporates a random term for cross-species variation (allometric scatter).

$$P(A, \theta_m, \theta_r, \theta_h \mid D_m, D_r, D_h)$$
$$\propto P(A)\, P(\theta_m \mid A)\, P(D_m \mid \theta_m)\, P(\theta_r \mid A)\, P(D_r \mid \theta_r)\, P(\theta_h \mid A)\, P(D_h \mid \theta_h) \quad \text{(Eq. A-21)}$$
$$\propto \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, \varphi(D_r - \theta_r, \sigma_r^2) \quad \text{(Eq. A-22)}$$
$$\varphi(\theta_h - A, \sigma_\varepsilon^2)\, \varphi(D_h - \theta_h, \sigma_h^2)$$

Consider marginalizing first over θ_m, then over θ_r, and then over A:

$$\int P(A, \theta_m, \theta_r, \theta_h \mid D_m, D_r, D_h)\, d\theta_m\, d\theta_r\, dA$$
$$\propto [\int P(A)\, \{\int P(\theta_m \mid A)\, P(D_m \mid \theta_m)\, d\theta_m\}\ \{\int P(\theta_r \mid A)\, P(D_r \mid \theta_r)\, d\theta_r\}\, P(\theta_h \mid A)\, dA \quad \text{(Eq. A-23)}$$
$$P(D_h \mid \theta_h)$$
$$= [\int P(A)\, P(D_m \mid A)\, P(D_r \mid A)\, P(\theta_h \mid A)\, dA\ ]\, P(D_h \mid \theta_h) \quad \text{(Eq. A-24)}$$
$$\propto [\int P(A \mid D_m\, D_r)\, P(\theta_h \mid A)\, dA]\, P(D_h \mid \theta_h) \quad \text{(Eq. A-25)}$$
$$= P(\theta_h \mid D_m\, D_r)\, P(D_h \mid \theta_h) \quad \text{(Eq. A-26)}$$

So $P(\theta_h \mid D_m\, D_r)$ is the prior for θ_h based on the mouse and rat data, and $P(D_h \mid \theta_h)$ as the human-specific likelihood. The prior is used in the MCMC analysis for the humans, and it is derived to be

$$P(\theta_h \mid D_m\, D_r) \propto \int \varphi(\theta_m - A, \sigma_\varepsilon^2)\, \varphi(D_m - \theta_m, \sigma_m^2)\, \varphi(\theta_r - A, \sigma_\varepsilon^2)\, \varphi(D_r - \theta_r, \sigma_r^2) \quad \text{(Eq. A-27)}$$

$$\varphi(\theta_h - A, \sigma_\varepsilon^2)\, d\theta_m\, d\theta_r\, dA$$

$$= \int [\varphi(D_m - A, \sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(D_r - A, \sigma_\varepsilon^2 + \sigma_r^2)]\, \varphi(\theta_h - A, \sigma_\varepsilon^2)\, dA \quad \text{(Eq. A-28)}$$

$$\propto \int \varphi(D_{m+r} - A, \sigma_{m+r}^2)\, \varphi(\theta_h - A, \sigma_\varepsilon^2)\, dA \quad \text{(Eq. A-29)}$$

$$= \varphi(D_{m+r} - \theta_h, \sigma_{m+r}^2 + \sigma_\varepsilon^2) \quad \text{(Eq. A-30)}$$

where D_{m+r} and σ_{m+r}^2 are the weighted mean and variances of A under the density

$$[\varphi(D_m - A, \sigma_\varepsilon^2 + \sigma_m^2)\, \varphi(D_r - A, \sigma_\varepsilon^2 + \sigma_r^2)] \quad \text{(Eq. A-31)}$$

which is given by

$$D_{m+r} = E(A \mid D_m\, D_r) = \{D_m/(\sigma_\varepsilon^2 + \sigma_m^2) + D_r/(\sigma_\varepsilon^2 + \sigma_r^2)\}/\{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}$$

$$= \text{weighted mean of } D_m \text{ and } D_r \quad \text{(Eq. A-32)}$$

$$\sigma_{m+r}^2 = \mathrm{VAR}(A \mid D_m\, D_r) = \{1/(\sigma_\varepsilon^2 + \sigma_m^2) + 1/(\sigma_\varepsilon^2 + \sigma_r^2)\}^{-1} \quad \text{(Eq. A-33)}$$

$$= \text{harmonic mean of variances}$$

At this point, these values are used in the normal approximation of the combined rodent posterior, which will be incorporated into the cross-species extrapolation as described in Step 5 above.

The results of these calculations for the updated prior distributions, are shown in Table A-6. With this methodology for updating the prior distributions, adequate convergence was achieved for the rat and human after 110,000~140,000 iterations.

A.4.2.3. *Population Variance: Prior Central Estimates and Uncertainty*

The following multipage Table A-7 describes the uncertainty distributions used for the population variability in the PBPK model parameters.

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters

Scaling (sampled) parameter	Mouse		Rat		Human		Notes/ source
	CV	CU	CV	CU	CV	CU	
Flows							
lnQCC	0.2	2	0.14	2	0.2	2	[a]
lnVPRC	0.2	2	0.3	2	0.2	2	
lnDRespC	0.2	0.5	0.2	0.5	0.2	0.5	
Physiological blood flows to tissues							
QFatC	0.46	0.5	0.46	0.5	0.46	0.5	[a]
QGutC	0.17	0.5	0.17	0.5	0.18	0.5	
QLivC	0.17	0.5	0.17	0.5	0.45	0.5	
QSlwC	0.29	0.5	0.3	0.5	0.32	0.5	
QKidC	0.32	0.5	0.13	0.5	0.12	0.5	
FracPlasC	0.2	0.5	0.2	0.5	0.05	0.5	
Physiological volumes							
VFatC	0.45	0.5	0.45	0.5	0.45	0.5	[a]
VGutC	0.13	0.5	0.13	0.5	0.08	0.5	
VLivC	0.24	0.5	0.18	0.5	0.23	0.5	
VRapC	0.1	0.5	0.12	0.5	0.08	0.5	
VRespLumC	0.11	0.5	0.18	0.5	0.2	0.5	
VRespEffC	0.11	0.5	0.18	0.5	0.2	0.5	
VKidC	0.1	0.5	0.15	0.5	0.17	0.5	
VBldC	0.12	0.5	0.12	0.5	0.12	0.5	
TCE distribution/partitioning							
lnPBC	0.25	2	0.25	0.333	0.185	0.333	[b]
lnPFatC	0.3	2	0.3	0.333	0.2	1	
lnPGutC	0.4	2	0.4	2	0.4	2	
lnPLivC	0.4	2	0.15	0.333	0.4	1.414	
lnPRapC	0.4	2	0.4	2	0.4	2	
lnPRespC	0.4	2	0.4	2	0.4	2	
lnPKidC	0.4	2	0.3	0.577	0.2	1.414	
lnPSlwC	0.4	2	0.3	0.333	0.3	1.414	
TCA distribution/partitioning							
lnPRBCPlasTCAC	0.336	2	0.336	2	0.336	2	[c]
lnPBodTCAC	0.336	2	0.693	2	0.336	2	[b]
lnPLivTCAC	0.336	2	0.693	2	0.336	2	
TCA plasma binding							
lnkDissocC	1.191	2	0.61	2	0.06	2	[b]
lnBMaxkDC	0.495	2	0.47	2	0.182	2	

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse		Rat		Human		Notes/ source
	CV	CU	CV	CU	CV	CU	
TCOH and TCOG distribution/partitioning							
lnPBodTCOHC	0.336	2	0.693	2	0.336	2	b
lnPLivTCOHC	0.336	2	0.693	2	0.336	2	b
lnPBodTCOGC	0.4	2	0.4	2	0.4	2	d
lnPLivTCOGC	0.4	2	0.4	2	0.4	2	d
DCVG distribution/partitioning							
lnPeffDCVG	0.4	2	0.4	2	0.4	2	b
TCE metabolism							
lnV_{MAX}C	0.824	1	0.806	1	0.708	0.26	e
lnK_MC	0.270	1	1.200	1			
lnClC					0.944	1.41	
lnFracOtherC	0.5	2	0.5	2	0.5	2	f
lnFracTCAC	0.5	2	0.5	2	1.8	2	g
lnV_{MAX}DCVGC	0.5	2	0.5	2			f
lnClDCVGC	0.5	2	0.5	2	0.5	2	
lnK_MDCVGC					0.5	2	
lnV_{MAX}KidDCVGC	0.5	2	0.5	2			
lnClKidDCVGC	0.5	2	0.5	2	0.5	2	
lnK_MKidDCVGC					0.5	2	
lnV_{MAX}LungLivC	0.5	2	0.5	2	0.5	2	
lnK_MClara	0.5	2	0.5	2	0.5	2	
lnFracLungSysC	0.5	2	0.5	2	0.5	2	
TCOH metabolism							
lnV_{MAX}TCOHC	0.5	2	0.5	2			f
lnClTCOHC					0.5	2	
lnK_MTCOH	0.5	2	0.5	2	0.5	2	
lnV_{MAX}GlucC	0.5	2	0.5	2			
lnClGlucC					0.5	2	
lnK_MGluc	0.5	2	0.5	2	0.5	2	
lnkMetTCOHC	0.5	2	0.5	2	0.5	2	
TCA metabolism/clearance							
lnkUrnTCAC	0.5	2	0.5	2	0.5	2	f
lnkMetTCAC	0.5	2	0.5	2	0.5	2	
TCOG metabolism/clearance							
lnkBileC	0.5	2	0.5	2	0.5	2	f
lnkEHRC	0.5	2	0.5	2	0.5	2	

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters (continued)

Scaling (sampled) parameter	Mouse		Rat		Human		Notes/ source
	CV	CU	CV	CU	CV	CU	
lnkUrnTCOGC	0.5	2	0.5	2	0.5	2	f
DCVG metabolism/clearance							
lnFracKidDCVCC	0.5	2	0.5	2	0.5	2	f
lnkDCVGC	0.5	2	0.5	2	0.5	2	
DCVC metabolism/clearance							
lnkNATC	0.5	2	0.5	2	0.5	2	f
lnkKidBioactC	0.5	2	0.5	2	0.5	2	
Oral uptake/transfer coefficients							
lnkTSD	2	2	2	2	2	2	h
lnkAS	2	2	2	2	2	2	
lnkTD	2	2	2	2	2	2	
lnkAD	2	2	2	2	2	2	
lnkASTCA	2	2	2	2	2	2	
lnkASTCOH	2	2	2	2	2	2	

Explanatory note. All population variance parameters (V_pname, for parameter "pname") have Inverse-Gamma distributions, with the expected value given by CV and coefficient of uncertainty given by CU (i.e., standard deviation of V_pname divided by expected value of V_pname) (notation the same as Hack et al. [2006]). Under these conditions, the Inverse-Gamma distribution has a shape parameter is given by $\alpha = 2 + 1/CU^2$ and scale parameter $\beta = (\alpha - 1)\ CV^2$. In addition, it should be noted that, under a normal distribution and a uniform prior distribution on the population variance, the posterior distribution for the variance given n data points with a sample variance s^2 is given by and Inverse-Gamma distribution with $\alpha = (n - 1)/2$ and $\beta = \alpha\ s^2$. Therefore, the "effective" number of data points is given by $n = 5 + 2/CU^2$ and the "effective" sample variance is $s^2 = CV^2\ \alpha/(\alpha - 1)$.

[a]For physiological parameters, CV values generally taken to be equal to the uncertainty SD in the population mean, most of which were based on variability between studies (i.e., not clear whether variability represents uncertainty or variability). Given this uncertainty, CU of 2 assigned to cardiac output and ventilation-perfusion, while CU of 0.5 assigned to the remaining physiological parameters.
[b]As discussed above, it is not clear whether interstudy variability is due to interindividual or assay variability, so the same central were assigned to the uncertainty in the population mean as to the central estimate of the population variance. In the cases were direct measurements were available, the CU for the uncertainty in the population variance is based on the actual sample *n*, with the derivation discussed in the notes preceding this table. Otherwise, a CU of 2 was assigned, reflecting high uncertainty.
[c]Used value from uncertainty in population in mean in rats for all species with high uncertainty.
[d]No data, so assumed CV of 0.4 with high uncertainty.
[e]For mice and rats, based on variability in results from Lipscomb et al. (1998a) and Elfarra et al. (1998) in microsomes. Since only pooled or mean values are available, CU of 1 was assigned (moderate uncertainty). For humans, based on variability in *individual* samples from Lipscomb et al. (1997) (microsomes), Elfarra et al. (1998) (microsomes) and Lipscomb et al. (1998a) (freshly isolated hepatocytes). High uncertainty in clearance (lnClC) reflects two different methods for scaling concentrations in microsomal preparations to blood concentrations: (1) assuming microsomal concentration equals liver concentration and then using the measured liver:blood partition coefficient to convert to blood and (2) using the measured microsome:air partition coefficient and then using the measured blood:air partition coefficient to convert to blood.
[f]No data on variability, so a CV of 0.5 was assigned, with a CU of 2.

Table A-7. Uncertainty distributions for the population variance of the PBPK model parameters (continued)

[g]For mice and rats, no data on variability, so a CV of 0.5 was assigned, with a CU of 2. For humans, 6-fold variability based on *in vitro* data from Bronley-DeLancy et al. (2006), but with high uncertainty.
[h]No data on variability, so a CV of 2 was assigned (larger than assumed for metabolism due to possible vehicle effects), with a CU of 2.

A.4.2.4. *Prior distributions for Residual Error Estimates*

In all cases except one, the likelihood was assumed to be lognormal, which requires specification of the variance of the "residual error." This error may include variability due to measurement error, intraindividual and intrastudy heterogeneity, as well as model misspecification. The available *in vivo* measurements to which the model was calibrated are listed in Table A-8. The variances for each of the corresponding residual errors were given log-uniform distributions. For all measurements, the bounds on the log-uniform distribution was 0.01 and 3.3, corresponding to geometric standard deviations bounded by 1.11 and 6.15. The lower bound was set to prevent "over-fitting," as was done in Bois (2000a) and Hack et al. (2006).

Nondetects of DCVG from Lash et al. (1999b) were also included in the data, at it was found that these data were needed to place constraints on the clearance rate of DCVG from blood. The detection limit reported in the study was LD = 0.05 pmol/mL= 5×10^{-5} mmol/L. It was assumed, as is standard in analytical chemistry, that the detection limit represents a response from a blank sample at 3-standard deviations. Because detector responses near the detection limit are generally normally distributed, the likelihood for observing a nondetect given a model-predicted value of y_p is equal to $P(\mathrm{ND}|y_p) = \Phi(3 \times \{1 - y_p/\mathrm{LD}\})$, where $\Phi(y)$ is the cumulative standard normal distribution.

The rat and human models differed from mouse model in terms of the hierarchical structure of the residual errors. In the mouse model, all the studies were assumed to have the same residual error, as shown in Figure A-1. This appeared reasonable because there were fewer studies, and there appeared to be less variation between studies. In the rat and human models, each of which used a much larger database of *in vivo* studies, residual errors were assumed to be the same within a study, but may differ between studies. The updated hierarchical structures are shown in Figure A-6. Initial attempts to use a single set of residual errors led to large residual errors for some measurements, even though fits to many studies appeared reasonable. Residual errors were generally reduced when study-specific errors were used, except for some datasets that appeared to be outliers (discussed below).

Table A-8. Measurements used for calibration

Measurement abbreviation	Mouse	Rat	Human	Measurement description
RetDose			√	Retained TCE dose (mg)
CAlvPPM			√	TCE concentration in alveolar air (ppm)
CInhPPM	√	√		TCE concentration in closed chamber (ppm)
CArt		√		TCE concentration in arterial blood (mg/L)
CVen	√	√	√	TCE concentration in venous blood (mg/L)
CBldMix	√	√		TCE concentration in mixed arterial and venous blood (mg/L)
CFat	√	√		TCE concentration in fat (mg/L)
CGut		√		TCE concentration in gut (mg/L)
CKid	√	√		TCE concentration in kidney (mg/L)
CLiv	√	√		TCE concentration in liver (mg/L)
CMus		√		TCE concentration in muscle (mg/L)
AExhpost	√	√		Amount of TCE exhaled postexposure (mg)
CTCOH	√	√	√	Free TCOH concentration in blood (mg/L)
CLivTCOH	√			Free TCOH concentration in liver (mg/L)
CPlasTCA	√	√	√	TCA concentration in plasma (mg/L)
CBldTCA	√	√	√	TCA concentration in blood (mg/L)
CLivTCA	√	√		TCA concentration in liver (mg/L)
AUrnTCA	√	√	√	Cumulative amount of TCA excreted in urine (mg)
AUrnTCA_collect			√	Cumulative amount of TCA collected in urine (noncontinuous sampling) (mg)
ABileTCOG		√		Cumulative amount of bound TCOH excreted in bile (mg)
CTCOG		√		Bound TCOH concentration in blood (mg/L)
CTCOGTCOH	√			Bound TCOH concentration in blood in free TCOH equivalents (mg/L)
CLivTCOGTCOH	√			Bound TCOH concentration in liver in free TCOH equivalents (mg/L)
AUrnTCOGTCOH	√	√	√	Cumulative amount of total TCOH excreted in urine (mg)
AUrnTCOGTCOH_ collect			√	Cumulative amount of total TCOH collected in urine (noncontinuous sampling) (mg)
CDCVGmol			√	DCVG concentration in blood (mmol/L)
CDCVG_ND			√	DCVG nondetects from Lash et al. (1999b)
AUrnNDCVC		√	√	Cumulative amount of NAcDCVC excreted in urine (mg)
AUrnTCTotMole		√		Cumulative amount of TCA+total TCOH excreted in urine (mmol)
TotCTCOH	√	√	√	Total TCOH concentration in blood (mg/L)

Rat Human

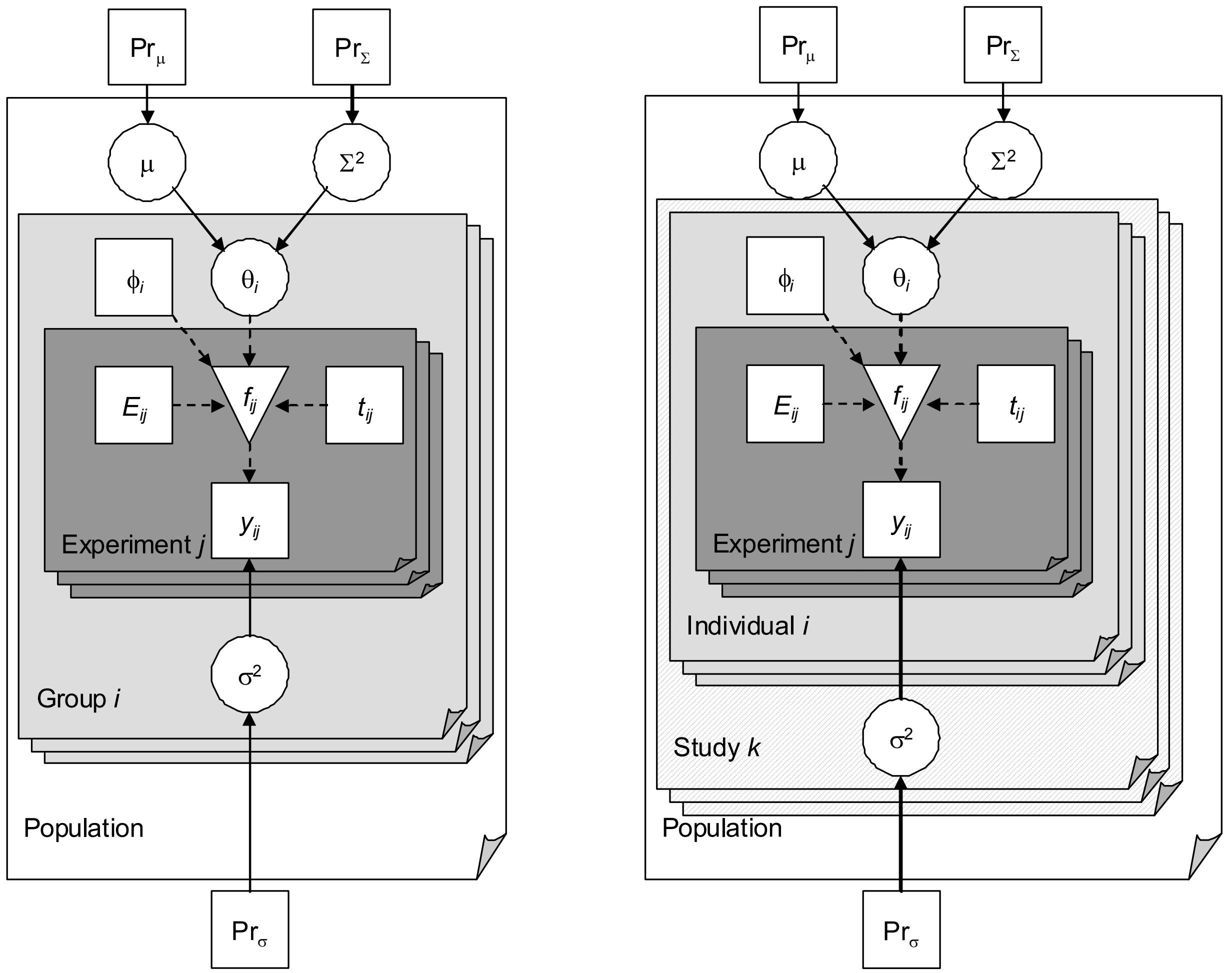

Figure A-6. Updated hierarchical structure for rat and human models. Symbols have the same meaning as Figure A-1, with modifications for the rat and human. In particular, in the rat, each "group" consists of animals (usually comprising multiple dose groups) of the same sex, species, and strain within a study (possibly reported in more than one publication, but reasonably presumed to be of animals in the same "lot"). Animals within each group are presumed to be "identical," with the same PBPK model parameters, and each such group is assigned its own set of "residual" error variances σ^2. In humans, each "individual" is a single person, possibly exposed in multiple experiments, and each individual is assigned a set of PBPK model parameters drawn from the population. However, in humans, "residual" error variances are assigned at the "study" level, rather than the individual or the population level.

A.5. RESULTS OF UPDATED PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL

The evaluation of the updated PBPK model was discussed in Chapter 3. Detailed results in the form of tables and figures are provided in this section.

A.5.1. Convergence and Posterior Distributions of Sampled Parameters

For each sampled parameter (population mean and variance and the variance for residual errors), summary statistics (median, [2.5%, 97.5%] confidence interval) for the posterior distribution are tabulated in Tables A-9−A-14 below. In addition, the potential scale reduction factor *R*, calculated from comparing four independent chains, is given.

In addition, posterior distributions for the group- or individual-specific parameters are summarized in supplementary figures accessible here:

- **Mouse:** Appendix.linked.files\AppA.5.1.Mouse.posteriors.by.group.pdf
- **Rat:** Appendix.linked.files\AppA.5.1.Rat.posteriors.by.group.pdf
- **Human:** Appendix.linked.files\AppA.5.1.Human.posteriors.by.group.or.individual.pdf.

A.5.2. Comparison of Model Predictions With Data

A.5.2.1. *Mouse Model*

A.5.2.1.1. ***Group-specific predictions and calibration data.*** [See Appendix.linked.files\AppA.5.2.1.1.Updated.mouse.group.calib.TCE.DRAFT.pdf.]

A.5.2.1.2. ***Population-based predictions and calibration data.*** [See Appendix.linked.files\AppA.5.2.1.2.Updated.mouse.pop.calib.TCE.DRAFT.pdf.]

A.5.2.2. *Rat Model*

A.5.2.2.1. ***Group-specific predictions and calibration data.*** [See Appendix.linked.files\AppA.5.2.2.1.Updated.rat.group.calib.TCE.DRAFT.pdf.]

A.5.2.2.2. ***Population-based predictions and calibration data.*** [See Appendix.linked.files\AppA.5.2.2.2.Updated.rat.pop.calib.TCE.DRAFT.pdf.]

A.5.2.2.3. ***Population-based predictions and additional evaluation data.*** [See Appendix.linked.files\AppA.5.2.2.3.Updated.rat.pop.eval.TCE.DRAFT.pdf.]

Table A-9. Posterior distributions for mouse PBPK model population parameters

Sampled parameter*	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population (geometric) standard deviation	
	Median (2.5%, 97.5%)	*R*	Median (2.5%, 97.5%)	*R*
lnQCC	1.237 (0.8972, 1.602)	1	1.402 (1.183, 2.283)	1
lnVPRC	0.8076 (0.6434, 1.022)	1	1.224 (1.108, 1.63)	1.001
QFatC	1.034 (0.5235, 1.55)	1	0.436 (0.3057, 0.6935)	1
QGutC	1.183 (1.002, 1.322)	1	0.1548 (0.1101, 0.2421)	1
QLivC	1.035 (0.8002, 1.256)	1	0.1593 (0.1107, 0.2581)	1
QSlwC	0.9828 (0.6043, 1.378)	1	0.275 (0.1915, 0.4425)	1
lnDRespC	1.214 (0.7167, 2.149)	1.002	1.215 (1.143, 1.375)	1
QKidC	0.995 (0.5642, 1.425)	1	0.3001 (0.21, 0.48)	1
FracPlasC	0.8707 (0.5979, 1.152)	1.001	0.1903 (0.1327, 0.3039)	1
VFatC	1.329 (0.8537, 1.784)	1.002	0.4123 (0.2928, 0.6414)	1
VGutC	0.9871 (0.817, 1.162)	1	0.1219 (0.085, 0.1965)	1
VLivC	0.8035 (0.5609, 1.093)	1.013	0.2216 (0.1552, 0.3488)	1
VRapC	0.997 (0.8627, 1.131)	1	0.09384 (0.06519, 0.1512)	1
VRespLumC	0.9995 (0.8536, 1.145)	1	0.1027 (0.07172, 0.1639)	1
VRespEffC	1 (0.8537, 1.148)	1.001	0.1032 (0.07176, 0.1652)	1
VKidC	1.001 (0.8676, 1.134)	1	0.09365 (0.06523, 0.1494)	1
VBldC	0.9916 (0.8341, 1.153)	1.001	0.1126 (0.07835, 0.1817)	1
lnPBC	0.9259 (0.647, 1.369)	1	1.644 (1.278, 3.682)	1
lnPFatC	0.9828 (0.7039, 1.431)	1.001	1.321 (1.16, 2.002)	1.001
lnPGutC	0.805 (0.4735, 1.418)	1	1.375 (1.198, 2.062)	1
lnPLivC	1.297 (0.7687, 2.039)	1	1.415 (1.21, 2.342)	1
lnPRapC	0.9529 (0.5336, 1.721)	1	1.378 (1.203, 2.141)	1
lnPRespC	0.9918 (0.5566, 1.773)	1.001	1.378 (1.2, 2.066)	1
lnPKidC	1.277 (0.7274, 2.089)	1	1.554 (1.265, 2.872)	1
lnPSlwC	0.92 (0.5585, 1.586)	1.001	1.411 (1.209, 2.3)	1.001
lnPRBCPlasTCAC	2.495 (1.144, 5.138)	1.001	1.398 (1.178, 2.623)	1.001
lnPBodTCAC	0.8816 (0.6219, 1.29)	1.003	1.27 (1.158, 1.609)	1
lnPLivTCAC	0.8003 (0.5696, 1.15)	1.003	1.278 (1.157, 1.641)	1.001
lnkDissocC	1.214 (0.2527, 4.896)	1.003	2.71 (1.765, 8.973)	1
lnBMaxkDC	1.25 (0.6793, 2.162)	1.002	1.474 (1.253, 2.383)	1
lnPBodTCOHC	0.8025 (0.5607, 1.174)	1	1.314 (1.17, 1.85)	1.001
lnPLivTCOHC	1.526 (0.9099, 2.245)	1	1.399 (1.194, 2.352)	1
lnPBodTCOGC	0.4241 (0.1555, 1.053)	1.004	1.398 (1.207, 2.156)	1
lnPLivTCOGC	1.013 (0.492, 2.025)	1.002	1.554 (1.279, 2.526)	1
lnPeffDCVG	0.9807 (0.008098, 149.6)	1.041	1.406 (1.206, 2.379)	1

Table A-9. Posterior distributions for mouse PBPK model population parameters (continued)

Sampled parameter*	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population (geometric) standard deviation	
	Median (2.5%, 97.5%)	*R*	Median (2.5%, 97.5%)	*R*
lnkTSD	5.187 (0.3909, 69.34)	1.001	5.858 (2.614, 80)	1
lnkAS	1.711 (0.3729, 11.23)	1.001	4.203 (2.379, 18.15)	1
lnkTD	0.1002 (0.01304, 0.7688)	1	5.16 (2.478, 60.24)	1
lnkAD	0.2665 (0.05143, 1.483)	1.003	4.282 (2.378, 20.21)	1
lnkASTCA	3.986 (0.1048, 141.9)	1	5.187 (2.516, 58.72)	1
lnkASTCOH	0.7308 (0.006338, 89.75)	1.001	5.047 (2.496, 54.8)	1
lnV_{MAX}C	0.6693 (0.4093, 1.106)	1.005	1.793 (1.49, 2.675)	1
lnK_MC	0.07148 (0.0323, 0.1882)	1	2.203 (1.535, 4.536)	1.001
lnFracOtherC	0.02384 (0.003244, 0.1611)	1.006	1.532 (1.265, 2.971)	1
lnFracTCAC	0.4875 (0.2764, 0.8444)	1.002	1.474 (1.258, 2.111)	1
lnV_{MAX}DCVGC	1.517 (0.02376, 1,421)	1.001	1.53 (1.263, 2.795)	1
lnClDCVGC	0.1794 (0.02333, 79.69)	1.013	1.528 (1.261, 2.922)	1
lnV_{MAX}KidDCVGC	1.424 (0.04313, 704.9)	1.014	1.533 (1.262, 2.854)	1
lnClKidDCVGC	0.827 (0.04059, 167.2)	1.019	1.527 (1.263, 2.874)	1
lnV_{MAX}LungLivC	2.903 (0.487, 12.1)	1.001	4.157 (1.778, 29.01)	1.018
lnK_MClara	0.01123 (0.001983, 0.09537)	1.012	1.629 (1.278, 5.955)	1.003
lnFracLungSysC	3.304 (0.2619, 182.1)	1.011	1.543 (1.266, 3.102)	1.001
lnV_{MAX}TCOHC	1.645 (0.6986, 3.915)	1.005	1.603 (1.28, 2.918)	1
lnK_MTCOH	0.9594 (0.2867, 2.778)	1.007	1.521 (1.264, 2.626)	1
lnV_{MAX}GlucC	65.59 (27.58, 232.5)	1.018	1.487 (1.254, 2.335)	1
lnK_MGluc	31.16 (6.122, 137.3)	1.015	1.781 (1.299, 5.667)	1.002
lnkMetTCOHC	3.629 (0.7248, 9.535)	1.009	1.527 (1.265, 2.626)	1
lnkUrnTCAC	0.1126 (0.04083, 0.2423)	1.012	1.757 (1.318, 3.281)	1.003
lnkMetTCAC	0.6175 (0.2702, 1.305)	1.027	1.508 (1.262, 2.352)	1.002
lnkBileC	0.9954 (0.316, 3.952)	1.003	1.502 (1.26, 2.453)	1
lnkEHRC	0.01553 (0.001001, 0.0432)	1.008	1.534 (1.264, 2.767)	1
lnkUrnTCOGC	7.874 (2.408, 50.28)	1	3.156 (1.783, 12.18)	1.001
lnFracKidDCVCC	1.931 (0.01084, 113.7)	1.018	1.53 (1.264, 2.77)	1
lnkDCVGC	0.2266 (0.001104, 16.46)	1.011	1.525 (1.263, 2.855)	1
lnkNATC	0.1175 (0.0008506, 14.34)	1.024	1.528 (1.264, 2.851)	1
lnkKidBioactC	0.07506 (0.0009418, 12.35)	1.035	1.527 (1.263, 2.84)	1.001

*These "sampled parameters" are scaled one or more times (see Table A-4) to obtain a biologically-meaningful parameter, posterior distributions of which are summarized in Tables 3-36 through 3-40). For natural log transformed parameters (name starting with "ln"), values are for the population geometric means and standard deviations.

Table A-10. Posterior distributions for mouse residual errors

Measurement	Residual error geometric standard deviation	
	Median (2.5%, 97.5%)	*R*
CInhPPM	1.177 (1.16, 1.198)	1.001
CVen	2.678 (2.354, 3.146)	1.001
CBldMix	1.606 (1.415, 1.96)	1.001
CFat	2.486 (2.08, 3.195)	1
CKid	2.23 (1.908, 2.796)	1
CLiv	1.712 (1.543, 1.993)	1
AExhpost	1.234 (1.159, 1.359)	1
CTCOH	1.543 (1.424, 1.725)	1
CLivTCOH	1.591 (1.454, 1.818)	1
CPlasTCA	1.396 (1.338, 1.467)	1.001
CBldTCA	1.488 (1.423, 1.572)	1.001
CLivTCA	1.337 (1.271, 1.43)	1
AUrnTCA	1.338 (1.259, 1.467)	1
CTCOGTCOH	1.493 (1.38, 1.674)	1.001
CLivTCOGTCOH	1.63 (1.457, 1.924)	1
AUrnTCOGTCOH	1.263 (1.203, 1.355)	1
TotCTCOH	1.846 (1.506, 2.509)	1.002

Note: the hierarchical statistical model for residual errors did not separate by group.

Table A-11. Posterior distributions for rat PBPK model population parameters

Sampled parameter	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population (geometric) standard deviation	
	Median (2.5%, 97.5%)	*R*	Median (2.5%, 97.5%)	*R*
lnQCC	1.195 (0.9285, 1.448)	1.034	1.298 (1.123, 2.041)	1.031
lnVPRC	0.6304 (0.4788, 0.8607)	1.012	1.446 (1.247, 2.011)	1.005
QFatC	1.167 (0.8321, 1.561)	1	0.4119 (0.2934, 0.6438)	1
QGutC	1.154 (0.988, 1.306)	1	0.1613 (0.1132, 0.2542)	1
QLivC	1.029 (0.8322, 1.223)	1.002	0.1551 (0.1092, 0.2483)	1
QSlwC	0.9086 (0.5738, 1.251)	1.001	0.2817 (0.1968, 0.4493)	1
lnDRespC	2.765 (1.391, 5.262)	1.018	1.21 (1.142, 1.358)	1.001
QKidC	1.002 (0.8519, 1.152)	1.001	0.1185 (0.08284, 0.1871)	1
FracPlasC	1.037 (0.8071, 1.259)	1.002	0.1785 (0.1272, 0.2723)	1
VFatC	0.9728 (0.593, 1.378)	1	0.4139 (0.2924, 0.6552)	1.002
VGutC	0.9826 (0.8321, 1.137)	1	0.1187 (0.08296, 0.1873)	1
VLivC	0.9608 (0.7493, 1.19)	1.015	0.1682 (0.1168, 0.2718)	1.001
VRapC	0.9929 (0.8563, 1.133)	1.001	0.1093 (0.07693, 0.175)	1
VRespLumC	1.001 (0.7924, 1.21)	1	0.1636 (0.116, 0.2601)	1
VRespEffC	0.999 (0.7921, 1.208)	1.001	0.1635 (0.1161, 0.2598)	1
VKidC	0.999 (0.8263, 1.169)	1	0.1361 (0.09617, 0.2167)	1
VBldC	1.002 (0.8617, 1.141)	1	0.1096 (0.07755, 0.176)	1
lnPBC	0.8551 (0.6854, 1.065)	1.001	1.317 (1.232, 1.462)	1.001
lnPFatC	1.17 (0.8705, 1.595)	1.003	1.333 (1.247, 1.481)	1.001
lnPGutC	0.8197 (0.5649, 1.227)	1	1.362 (1.198, 1.895)	1
lnPLivC	1.046 (0.8886, 1.234)	1.001	1.152 (1.115, 1.214)	1
lnPRapC	1.021 (0.6239, 1.675)	1.002	1.373 (1.201, 1.988)	1
lnPRespC	0.993 (0.5964, 1.645)	1.001	1.356 (1.197, 1.948)	1
lnPKidC	0.9209 (0.6728, 1.281)	1	1.304 (1.201, 1.536)	1
lnPSlwC	1.258 (0.9228, 1.711)	1.001	1.364 (1.263, 1.544)	1
lnPRBCPlasTCAC	0.9763 (0.6761, 1.353)	1	1.276 (1.159, 1.634)	1
lnPBodTCAC	1.136 (0.6737, 1.953)	1.008	1.631 (1.364, 2.351)	1.003
lnPLivTCAC	1.283 (0.6425, 2.491)	1.008	1.651 (1.356, 2.658)	1
lnkDissocC	1.01 (0.5052, 2.017)	1.002	1.596 (1.315, 2.774)	1
lnBMaxkDC	0.9654 (0.5716, 1.733)	1.02	1.412 (1.234, 2.01)	1
lnPBodTCOHC	0.9454 (0.4533, 1.884)	1.045	1.734 (1.39, 3.151)	1.002
lnPLivTCOHC	0.926 (0.3916, 2.196)	1.013	1.785 (1.382, 4.142)	1.003
lnPBodTCOGC	1.968 (0.09185, 14.44)	1.031	1.414 (1.208, 2.571)	1
lnPLivTCOGC	7.484 (2.389, 26.92)	1.017	1.41 (1.208, 2.108)	1
lnkTSD	3.747 (0.2263, 62.58)	1.01	6.777 (2.844, 87.29)	1

Table A-11. Posterior distributions for rat PBPK model population parameters (continued)

Sampled parameter	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population (geometric) standard deviation	
	Median (2.5%, 97.5%)	*R*	Median (2.5%, 97.5%)	*R*
lnkAS	2.474 (0.2542, 28.35)	1.004	10.16 (4.085, 143.7)	1
lnkAD	0.1731 (0.04001, 0.7841)	1.018	4.069 (2.373, 14.19)	1.009
lnkASTCA	1.513 (0.1401, 17.19)	1.002	4.376 (2.43, 22.83)	1
lnkASTCOH	0.6896 (0.01534, 25.81)	1.001	4.734 (2.444, 35.2)	1.001
lnV$_{MAX}$C	0.8948 (0.6377, 1.293)	1.028	1.646 (1.424, 2.146)	1.021
lnK$_{M}$C	0.0239 (0.01602, 0.04993)	1.001	2.402 (1.812, 4.056)	1.001
lnFracOtherC	0.344 (0.0206, 1.228)	1.442	3 (1.332, 10.04)	1.353
lnFracTCAC	0.2348 (0.122, 0.4616)	1.028	1.517 (1.264, 2.393)	1.001
lnV$_{MAX}$DCVGC	7.749 (0.2332, 458.8)	1.088	1.534 (1.262, 2.804)	1.001
lnClDCVGC	0.3556 (0.06631, 2.242)	1.018	1.509 (1.261, 2.553)	1
lnV$_{MAX}$KidDCVGC	0.2089 (0.04229, 1.14)	1.011	1.542 (1.263, 2.923)	1.001
lnClKidDCVGC	184 (26.29, 1312)	1.02	1.527 (1.265, 2.873)	1.001
lnV$_{MAX}$LungLivC	2.673 (0.4019, 14.16)	1.002	4.833 (1.599, 48.32)	1.002
lnK$_{M}$Clara	0.02563 (0.005231, 0.197)	1.01	1.66 (1.279, 18.74)	1.002
lnFracLungSysC	2.729 (0.04124, 63.27)	1.027	1.536 (1.267, 2.868)	1.001
lnV$_{MAX}$TCOHC	1.832 (0.6673, 6.885)	1.041	1.667 (1.292, 3.148)	1.002
lnK$_{M}$TCOH	22.09 (3.075, 131.9)	1.186	1.629 (1.276, 3.773)	1.017
lnV$_{MAX}$GlucC	28.72 (10.02, 86.33)	1.225	2.331 (1.364, 5.891)	1.126
lnK$_{M}$Gluc	6.579 (1.378, 23.57)	1.119	2.046 (1.309, 10.3)	1.125
lnkMetTCOHC	2.354 (0.3445, 15.83)	1.287	1.876 (1.283, 11.82)	1.182
lnkUrnTCAC	0.07112 (0.03934, 0.1329)	1.076	1.513 (1.27, 2.327)	1.003
lnkMetTCAC	0.3554 (0.1195, 0.8715)	1.036	1.528 (1.263, 2.444)	1.001
lnkBileC	8.7 (1.939, 26.71)	1.05	1.65 (1.282, 5.494)	1.017
lnkEHRC	1.396 (0.2711, 6.624)	1.091	1.647 (1.277, 5.582)	1.005
lnkUrnTCOGC	20.65 (2.437, 138)	1.041	1.595 (1.269, 5.257)	1.026
lnkNATC	0.002035 (0.0004799, 0.01019)	1.01	1.523 (1.261, 2.593)	1.001
lnkKidBioactC	0.006618 (0.0009409, 0.0367)	1.039	1.52 (1.261, 2.674)	1

Table A-12. Posterior distributions for rat residual errors

Measurement	Group	Residual error geometric standard deviation	
		Median (2.5%, 97.5%)	*R*
CInhPPM	Group 3	1.124 (1.108, 1.147)	1
	Group 16	1.106 (1.105, 1.111)	1
CMixExh	Group 2	1.501 (1.398, 1.65)	1
CArt	Group 2	1.174 (1.142, 1.222)	1
	Group 6	1.523 (1.321, 1.918)	1.002
CVen	Group 4	1.22 (1.111, 1.877)	1
	Group 7	1.668 (1.489, 1.986)	1.001
	Group 8	1.45 (1.234, 2.065)	1.014
	Group 9	1.571 (1.426, 1.811)	1
	Group 10	4.459 (2.754, 6.009)	1
	Group 11	1.587 (1.347, 2.296)	1.002
	Group 16	1.874 (1.466, 2.964)	1.011
	Group 18	1.676 (1.188, 3.486)	1.003
CBldMix	Group 12	1.498 (1.268, 2.189)	1
CFat	Group 9	1.846 (1.635, 2.184)	1
	Group 16	2.658 (1.861, 4.728)	1.001
CGut	Group 9	1.855 (1.622, 2.243)	1
CKid	Group 9	1.469 (1.354, 1.648)	1
CLiv	Group 9	1.783 (1.554, 2.157)	1
	Group 12	1.744 (1.401, 2.892)	1
	Group 16	1.665 (1.376, 2.411)	1.001
CMus	Group 9	1.653 (1.494, 1.919)	1
AExhpost	Group 6	1.142 (1.108, 1.239)	1.003
	Group 10	1.117 (1.106, 1.184)	1.004
	Group 14	1.166 (1.107, 1.475)	1
	Group 15	1.125 (1.106, 1.237)	1
CTCOH	Group 6	1.635 (1.455, 1.983)	1.002
	Group 10	1.259 (1.122, 1.868)	1.009
	Group 11	1.497 (1.299, 1.923)	1.01
	Group 13	1.611 (1.216, 3.556)	1.001
	Group 17	1.45 (1.213, 2.208)	1.004
	Group 18	1.142 (1.107, 1.268)	1
CPlasTCA	Group 4	1.134 (1.106, 1.254)	1
	Group 5	1.141 (1.107, 1.291)	1
	Group 11	1.213 (1.136, 1.381)	1
	Group 19	1.201 (1.145, 1.305)	1

Table A-12. Posterior distributions for rat residual errors (continued)

Measurement	Group	Residual error geometric standard deviation	
		Median (2.5%, 97.5%)	*R*
CBldTCA	Group 4	1.134 (1.106, 1.258)	1
	Group 5	1.14 (1.107, 1.289)	1
	Group 6	1.59 (1.431, 1.878)	1.001
	Group 11	1.429 (1.292, 1.701)	1.001
	Group 17	1.432 (1.282, 1.675)	1.03
	Group 18	1.193 (1.12, 1.358)	1.004
	Group 19	1.214 (1.153, 1.327)	1
CLivTCA	Group 19	1.666 (1.443, 2.104)	1
AUrnTCA	Group 1	1.498 (1.125, 2.18)	1.135
	Group 6	1.95 (1.124, 5.264)	1.003
	Group 8	1.221 (1.146, 1.375)	1.003
	Group 10	1.18 (1.108, 1.444)	1.007
	Group 17	1.753 (1.163, 4.337)	1.001
	Group 19	1.333 (1.201, 1.707)	1
ABileTCOG	Group 6	2.129 (1.128, 5.363)	1.003
CTCOG	Group 17	2.758 (1.664, 5.734)	1.028
AUrnTCOGTCOH	Group 1	1.129 (1.106, 1.232)	1.004
	Group 6	1.483 (1.113, 4.791)	1.002
	Group 8	1.115 (1.106, 1.162)	1
	Group 10	1.145 (1.107, 1.305)	1
	Group 17	2.27 (1.53, 4.956)	1.009
AUrnNDCVC	Group 1	1.168 (1.11, 1.33)	1.002
AUrnTCTotMole	Group 6	1.538 (1.182, 3.868)	1.002
	Group 7	1.117 (1.106, 1.153)	1.001
	Group 14	1.121 (1.106, 1.207)	1
	Group 15	1.162 (1.108, 1.358)	1
TotCTCOH	Group 17	1.488 (1.172, 2.366)	1.015

The nineteen groups are (1) Bernauer et al., 1996; (2) Dallas et al., 1991; (3) Fisher et al., 1989 females; (4) Fisher et al., 1991 females; (5) Fisher et al., 1991 males; (6) Green and Prout, 1985, Prout et al., 1985, male OA rats; (7) Hissink et al., 2002; (8) Kaneko et al., 1994; (9) Keys et al., 2003; (10) Kimmerle and Eben, 1973a; (11) Larson and Bull, 1992a, b; (12) Lee et al., 2000; (13) Merdink et al., 1999; (14) Prout et al., 1985 AP rats; (15) Prout et al., 1985 OM rats; (16) Simmons et al., 2002; (17) Stenner et al., 1997; (18) Templin et al., 1995; (19) Yu et al., 2000.

Table A-13. Posterior distributions for human PBPK model population parameters

Sampled parameter	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population (geometric) standard deviation	
	Median (2.5%, 97.5%)	*R*	Median (2.5%, 97.5%)	*R*
lnQCC	0.837 (0.6761, 1.022)	1.038	1.457 (1.271, 1.996)	1.036
lnVPRC	1.519 (1.261, 1.884)	1.007	1.497 (1.317, 1.851)	1.008
QFatC	0.7781 (0.405, 1.143)	1.014	0.6272 (0.4431, 0.9773)	1
QGutC	0.7917 (0.6631, 0.925)	1.017	0.1693 (0.1199, 0.2559)	1.019
QLivC	0.5099 (0.1737, 0.8386)	1.031	0.4167 (0.2943, 0.6324)	1.009
QSlwC	0.7261 (0.4864, 0.9234)	1.011	0.3166 (0.2254, 0.4802)	1.005
lnDRespC	0.626 (0.3063, 1.013)	1.197	1.291 (1.158, 2.006)	1.083
QKidC	1.007 (0.9137, 1.103)	1.009	0.1004 (0.07307, 0.1545)	1
FracPlasC	1.001 (0.9544, 1.047)	1.01	0.04275 (0.03155, 0.06305)	1
VFatC	0.788 (0.48, 1.056)	1.005	0.3666 (0.2696, 0.5542)	1
VGutC	1 (0.937, 1.067)	1.007	0.06745 (0.04923, 0.1038)	1
VLivC	1.043 (0.8683, 1.23)	1.047	0.1959 (0.1424, 0.3017)	1.003
VRapC	0.9959 (0.9311, 1.06)	1.006	0.06692 (0.04843, 0.1027)	1
VRespLumC	1.003 (0.8461, 1.164)	1.001	0.1671 (0.1209, 0.255)	1
VRespEffC	1 (0.8383, 1.159)	1.001	0.1672 (0.1215, 0.259)	1
VKidC	0.9965 (0.8551, 1.14)	1.007	0.1425 (0.1037, 0.2183)	1
VBldC	1.013 (0.9177, 1.108)	1.003	0.1005 (0.07265, 0.1564)	1
lnPBC	0.9704 (0.8529, 1.101)	1.001	1.216 (1.161, 1.307)	1.002
lnPFatC	0.8498 (0.7334, 0.9976)	1.002	1.188 (1.113, 1.366)	1.002
lnPGutC	1.095 (0.7377, 1.585)	1.029	1.413 (1.214, 2.05)	1.002
lnPLivC	0.9907 (0.6679, 1.441)	1.01	1.338 (1.203, 1.683)	1
lnPRapC	0.93 (0.6589, 1.28)	1.003	1.528 (1.248, 2.472)	1.001
lnPRespC	1.018 (0.6773, 1.5)	1.015	1.32 (1.192, 1.656)	1
lnPKidC	0.9993 (0.8236, 1.219)	1.003	1.155 (1.097, 1.287)	1
lnPSlwC	1.157 (0.8468, 1.59)	1.018	1.69 (1.383, 3.157)	1.008
lnPRBCPlasTCAC	0.3223 (0.04876, 0.8378)	1.007	5.507 (3.047, 19.88)	1.003
lnPBodTCAC	1.194 (0.929, 1.481)	1.043	1.327 (1.185, 1.67)	1.018
lnPLivTCAC	1.202 (0.8429, 1.634)	1.046	1.285 (1.162, 1.648)	1.007
lnkDissocC	0.9932 (0.9387, 1.053)	1.012	1.043 (1.026, 1.076)	1.003
lnBMaxkDC	0.8806 (0.7492, 1.047)	1.038	1.157 (1.085, 1.37)	1.012
lnPBodTCOHC	1.703 (1.439, 2.172)	1.019	1.409 (1.267, 1.678)	1.011
lnPLivTCOHC	1.069 (0.7643, 1.485)	1.028	1.288 (1.165, 1.629)	1.002
lnPBodTCOGC	0.7264 (0.1237, 2.54)	1.003	11.98 (5.037, 185.3)	1.017
lnPLivTCOGC	6.671 (1.545, 24.87)	1.225	5.954 (2.653, 23.68)	1.052
lnPeffDCVG	0.01007 (0.003264, 0.03264)	1.004	1.385 (1.201, 2.03)	1.001

1

Table A-13. Posterior distributions for human PBPK model population parameters (continued)

Sampled parameter	Posterior distributions reflecting uncertainty in population distribution			
	Population (geometric) mean		Population (geometric) standard deviation	
	Median (2.5%, 97.5%)	*R*	Median (2.5%, 97.5%)	*R*
lnkASTCA	4.511 (0.04731, 465.7)	1	5.467 (2.523, 71.06)	1
lnkASTCOH	8.262 (0.0677, 347.9)	1	5.481 (2.513, 67.86)	1
lnV_{MAX}C	0.3759 (0.2218, 0.5882)	1.026	2.21 (1.862, 2.848)	1.003
lnClC	12.64 (5.207, 39.96)	1.028	4.325 (2.672, 9.003)	1.016
lnFracOtherC	0.1186 (0.02298, 0.2989)	1.061	3.449 (1.392, 9.146)	1.102
lnFracTCAC	0.1315 (0.07115, 0.197)	1.026	2.467 (1.916, 3.778)	1.01
lnClDCVGC	2.786 (1.326, 5.769)	1.08	2.789 (1.867, 4.877)	1.02
lnK_MDCVGC	1.213 (0.3908, 4.707)	1.029	4.43 (2.396, 18.56)	1.035
lnClKidDCVGC	0.04538 (0.001311, 0.1945)	1.204	3.338 (1.295, 30.46)	1.095
lnK_MKidDCVGC	0.2802 (0.1096, 1.778)	1.097	1.496 (1.263, 2.317)	1.001
lnV_{MAX}LungLivC	3.772 (0.8319, 9.157)	1.035	2.228 (1.335, 21.89)	1.014
lnK_MClara	0.2726 (0.02144, 1.411)	1.041	11.63 (1.877, 682.7)	1.041
lnFracLungSysC	24.08 (6.276, 81.14)	1.016	1.496 (1.263, 2.439)	1.001
lnClTCOHC	0.1767 (0.1374, 0.2257)	1.011	1.888 (1.624, 2.307)	1.01
lnK_MTCOH	2.221 (1.296, 4.575)	1.02	2.578 (1.782, 4.584)	1.015
lnClGlucC	0.2796 (0.2132, 0.3807)	1.056	1.955 (1.583, 2.418)	1.079
lnK_MGluc	133.4 (51.56, 277.2)	1.02	1.573 (1.266, 4.968)	1.011
lnkMetTCOHC	0.7546 (0.1427, 2.13)	1.007	5.011 (2.668, 15.71)	1.002
lnkUrnTCAC	0.04565 (0.0324, 0.06029)	1.005	1.878 (1.589, 2.48)	1.006
lnkMetTCAC	0.2812 (0.1293, 0.5359)	1.004	2.529 (1.78, 4.211)	1.002
lnkBileC	6.855 (3.016, 20.69)	1.464	1.589 (1.27, 3.358)	1.015
lnkEHRC	0.1561 (0.09511, 0.2608)	1.1	1.699 (1.348, 2.498)	1.015
lnkUrnTCOGC	15.78 (6.135, 72.5)	1.007	9.351 (4.93, 29.96)	1.003
lnkDCVGC	7.123 (5.429, 9.702)	1.026	1.507 (1.311, 1.897)	1.008
lnkNATC	0.0003157 (0.0001087, 0.002305)	1.008	1.54 (1.261, 3.306)	1
lnkKidBioactC	0.06516 (0.01763, 0.1743)	1.001	1.523 (1.262, 2.987)	1

Table A-14. Posterior distributions for human residual errors

Measurement	Group	Residual error geometric standard deviation	
		Median (2.5%, 97.5%)	*R*
RetDose	Group 4	1.131 (1.106, 1.25)	1.001
CAlvPPM	Group 1	1.832 (1.509, 2.376)	1.015
	Group 4	1.515 (1.378, 1.738)	1
	Group 5	1.44 (1.413, 1.471)	1
CVen	Group 1	1.875 (1.683, 2.129)	1.018
	Group 3	1.618 (1.462, 1.862)	1
	Group 4	1.716 (1.513, 2.057)	1.001
	Group 5	2.948 (2.423, 3.8)	1.007
CTCOH	Group 1	1.205 (1.185, 1.227)	1.012
	Group 3	1.213 (1.187, 1.247)	1
	Group 5	2.101 (1.826, 2.571)	1.001
	Group 7	1.144 (1.106, 2.887)	1.123
CPlasTCA	Group 2	1.117 (1.106, 1.17)	1.001
	Group 7	1.168 (1.123, 1.242)	1
CBldTCA	Group 1	1.138 (1.126, 1.152)	1.003
	Group 2	1.119 (1.106, 1.178)	1
	Group 4	1.488 (1.351, 1.646)	1.018
	Group 5	1.438 (1.367, 1.537)	1.002
zAUrnTCA	Group 1	1.448 (1.414, 1.485)	1.001
	Group 2	1.113 (1.105, 1.149)	1.001
	Group 3	1.242 (1.197, 1.301)	1.001
	Group 4	1.538 (1.441, 1.67)	1
	Group 6	1.158 (1.118, 1.228)	1
	Group 7	1.119 (1.106, 1.181)	1
zAUrnTCA_collect	Group 3	1.999 (1.178, 3.903)	1.003
	Group 5	2.787 (2.134, 4.23)	1.001
AUrnTCOGTCOH	Group 1	1.106 (1.105, 1.112)	1.001
	Group 3	1.11 (1.105, 1.125)	1
	Group 4	1.124 (1.107, 1.151)	1.001
	Group 6	1.117 (1.106, 1.157)	1.001
	Group 7	1.134 (1.106, 1.348)	1.003
AUrnTCOGTCOH_collect	Group 3	1.3 (1.111, 2.333)	1.004
	Group 5	1.626 (1.524, 1.767)	1
CDCVGmol	Group 1	1.53 (1.436, 1.656)	1.009
zAUrnNDCVC	Group 6	1.167 (1.124, 1.244)	1
TotCTCOH	Group 1	1.204 (1.185, 1.226)	1.011
	Group 4	1.247 (1.177, 1.366)	1.009
	Group 5	1.689 (1.552, 1.9)	1.001

The seven groups are (1) Fisher et al., 1998; (2) Paycok and Powell, 1945; (3) Kimmerle and Eben, 1973b; (4) Monster et al., 1976; (5) Chiu et al., 2007; (6) Bernauer et al., 1996; (7) Muller et al., 1974.

A.5.2.3. *Human Model*

A.5.2.3.1. *Individual-specific predictions and calibration data.* [See Appendix.linked.files\AppA.5.2.3.1.Updated.human.indiv.calib.TCE.DRAFT.pdf.]

A.5.2.3.2. *Population-based predictions and calibration data.* [See Appendix.linked.files\AppA.5.2.3.2.Updated.human.pop.calib.TCE.DRAFT.pdf.]

A.5.2.3.3. *Population-based predictions and additional evaluation data.* [See Appendix.linked.files\AppA.5.2.3.3.Updated.human.pop.eval.TCE.DRAFT.pdf.]

A.6. EVALUATION OF RECENTLY PUBLISHED TOXICOKINETIC DATA

Several *in vivo* toxicokinetic studies were published or became available during internal U.S. EPA review and Interagency Consultation, and were not evaluated as part of the originally planned analyses. Preliminary analyses of these data are summarized here. The general approach is the same as that used for the evaluation data in the primary analysis—population predictions from the PBPK model are compared visually with the toxicokinetic data. Figures with the population-based predictions and these recently published data are in the following linked files:

- **Mouse (Kim et al., 2009; Mahle et al., 2001; Green, 2003a, b):** Appendix.linked.files\AppA.6.Updated.mouse.pop.eval.TCE.DRAFT.pdf.
- **Rat (Liu et al., 2009; Mahle et al., 2001):** Appendix.linked.files\AppA.6.Updated.rat.pop.eval.TCE.DRAFT.pdf.

A.6.1. TCE Metabolite Toxicokinetics in Mice: Kim et al. (2009)

Kim et al. (2009) measured TCA, DCA, DCVG, and DCVC in blood of male B6C3F1 mice following a single gavage dose of 2,140 mg/kg. Of these data, only TCA and DCVG blood concentrations are predicted by the updated PBPK model, so only those data are compared with PBPK model predictions (prior values for the distribution volume and elimination rate constant of DCVG were used, as there were no calibration data informing those parameters). These data were within the inter-quartile region of the PBPK model population predictions.

An assessment was made as to whether these data are informative as to the flux of GSH conjugation in mice. First, the best fitting parameter sample (least squares on TCA and DCVG in blood, weighted by inverse of the observed variance) from the posterior distribution was selected out of 50,000 samples generated by Monte Carlo (see Figures A-7 and A-8 for the comparison with predictions with data). This parameter sample was then used to calculate the fraction of intake that is predicted by the PBPK model to undergo GSH metabolism for continuous oral and continuous inhalation exposure, and this point estimate compared to the full posterior distribution (see Figures A-9 and A-10). The predictions for this "best fitting" parameter set was similar (within 3-fold) of the median of the full posterior distribution. While a formal assessment of the impact of these new data (i.e., including its uncertainty and variability) would require a re-running of the Bayesian analysis, it appears that the median estimates for the mouse GSH conjugation dose metric used in the dose-response assessment (see Chapter 5) are reasonably consistent with the Kim et al. (2009) data.

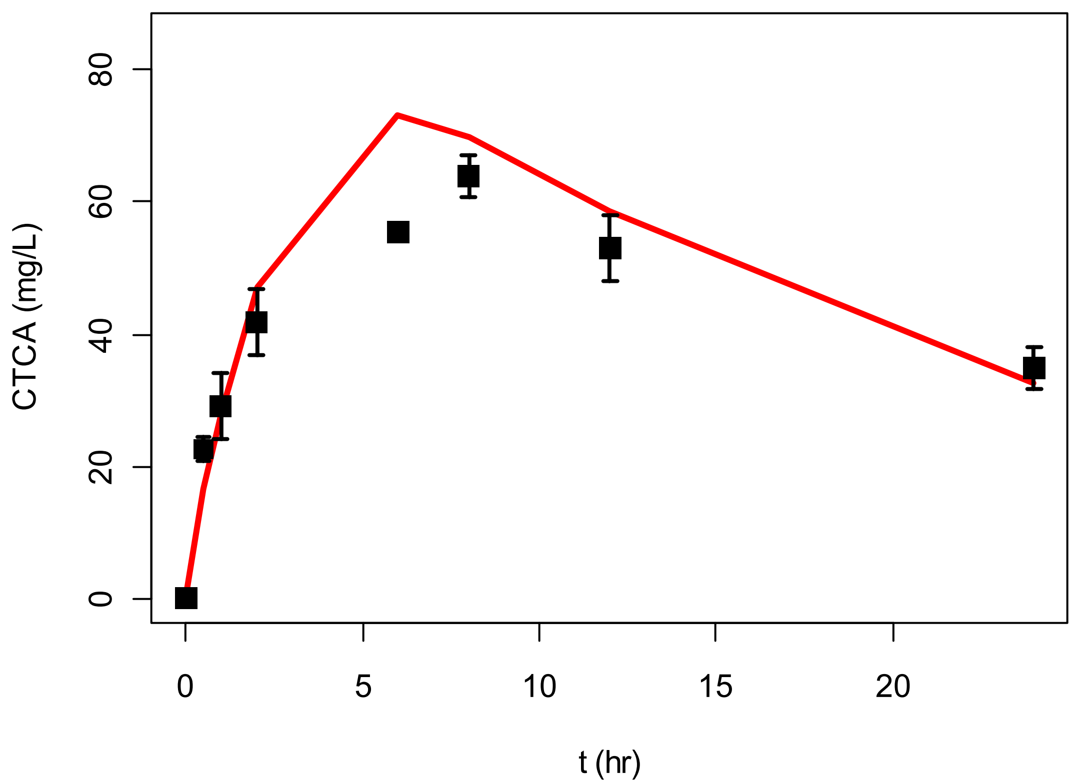

Figure A-7. Comparison of best-fitting (out of 50,000 posterior samples) PBPK model prediction and Kim et al. (2009) TCA blood concentration data for mice gavaged with 2,140 mg/kg TCE. Full population distributions are shown in a separate linked file (see text).

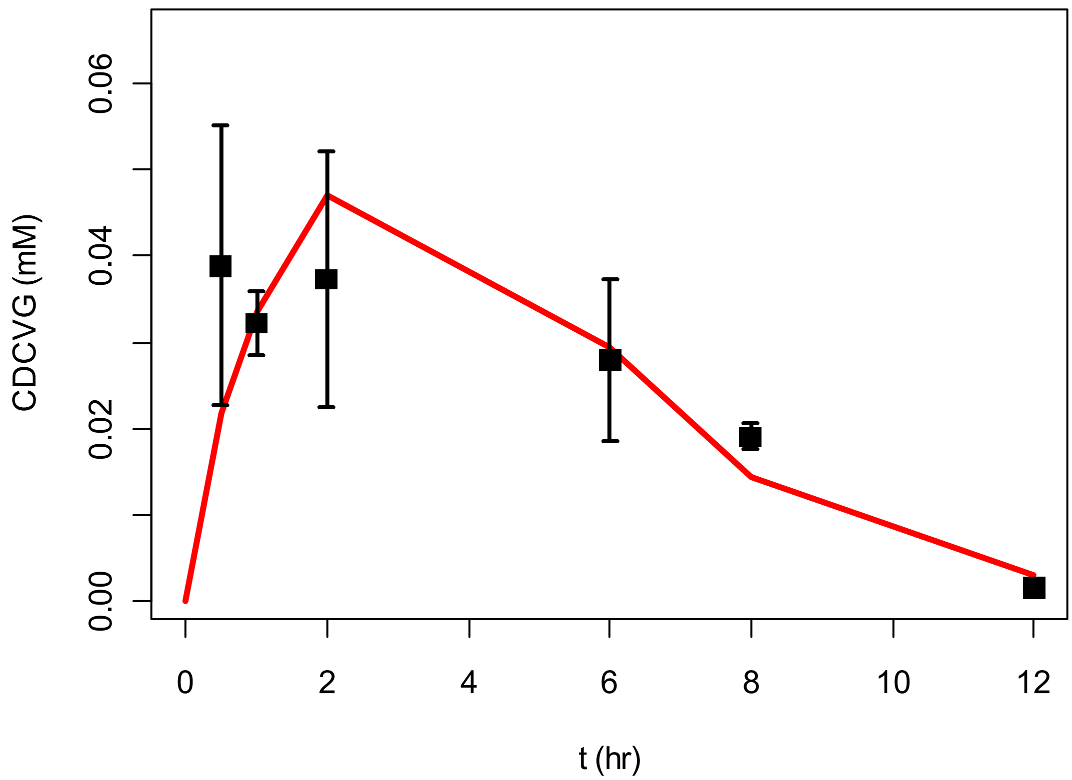

Figure A-8. Comparison of best-fitting (out of 50,000 posterior samples) PBPK model prediction and Kim et al. (2009) DCVG blood concentration data for mice gavaged with 2,140 mg/kg TCE. Full population distributions are shown in a separate linked file (see text).

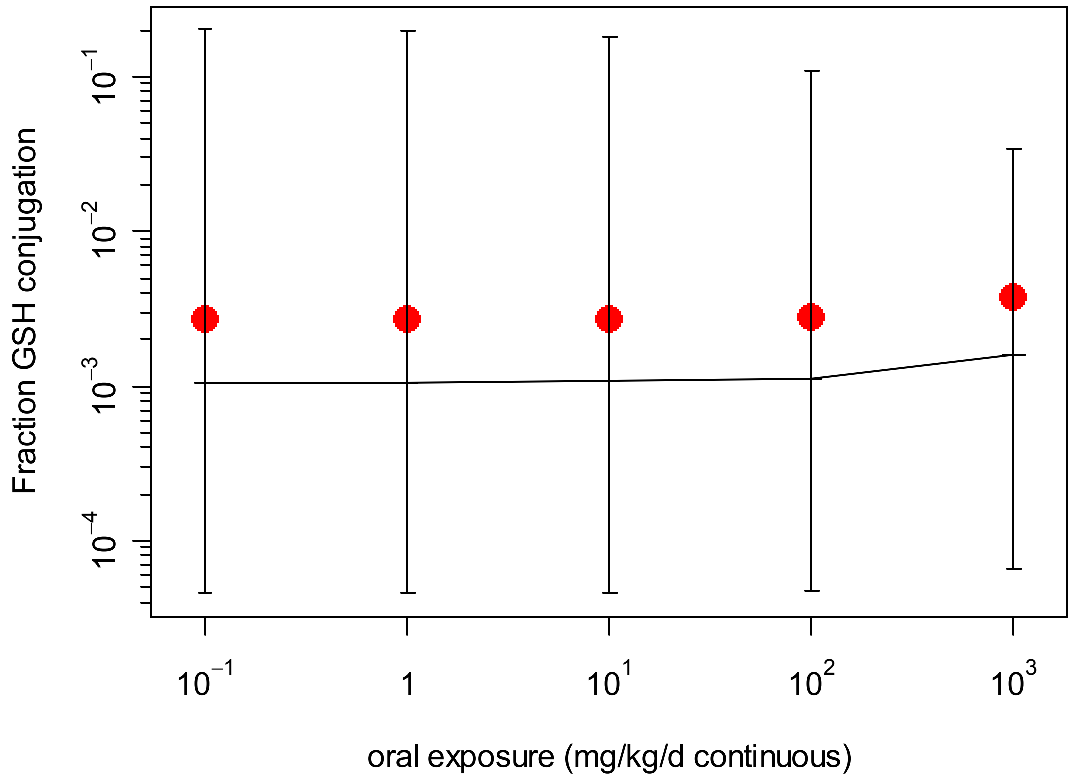

Figure A-9. PBPK model predictions for the fraction of intake undergoing GSH conjugation in mice continuously exposed orally to TCE. Lines and error bars represent the median and 95th percentile confidence interval for the posterior predictions, respectively (also reported in Section 3.5.7.2.1). Filled circles represent the predictions from the sample (out of 50,000 total posterior samples) which provides the best fit to the Kim et al. (2009) TCA and DCVG blood concentration data for mice gavaged with 2,140 mg/kg TCE.

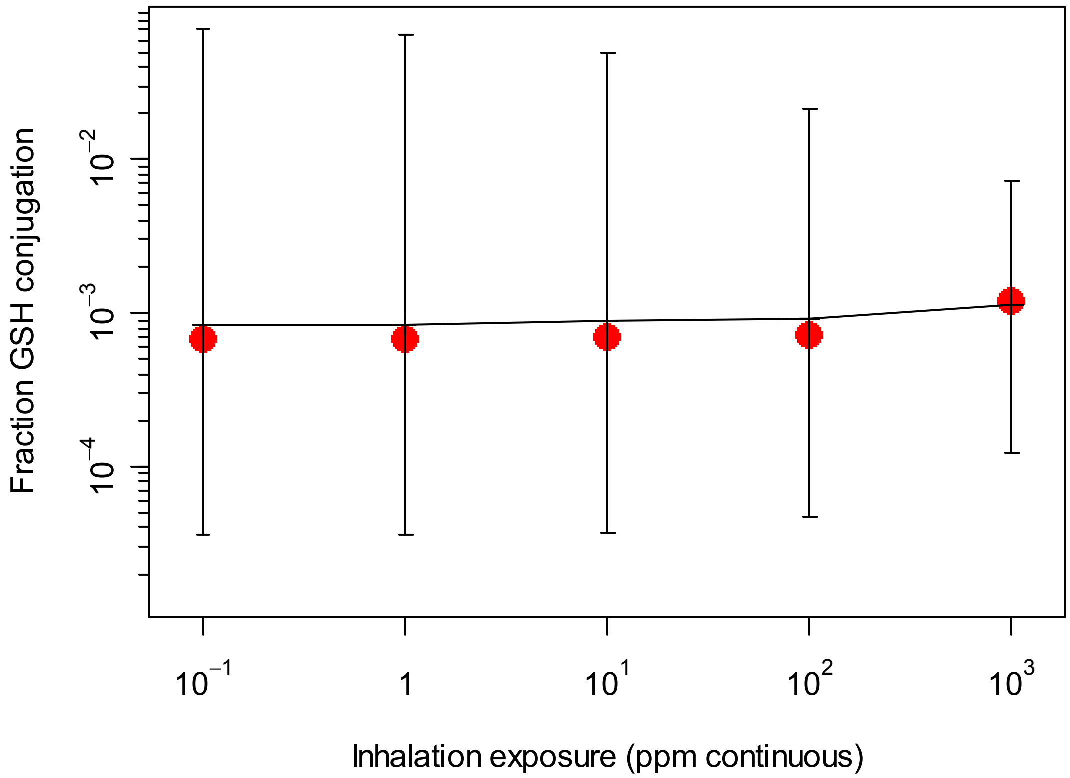

Figure A-10. PBPK model predictions for the fraction of intake undergoing GSH conjugation in mice continuously exposed via inhalation to TCE. Lines and error bars represent the median and 95th percentile confidence interval for the posterior predictions, respectively (also reported in Section 3.5.7.2.1). Filled circles represent the predictions from the sample (out of 50,000 total posterior samples) which provides the best fit to the Kim et al. (2009) TCA and DCVG blood concentration data for mice gavaged with 2,140 mg/kg TCE.

An additional note of interest from the Kim et al. (2009) data is the inter-study variability in TCA kinetics. In particular, the TCA blood concentrations reported by Kim et al. (2009) are 2-fold lower than those reported by Abbas and Fisher (1997) in the same sex and strain of mouse, with a very similar corn oil gavage dose of 2,000 mg/kg (as compared to 2,140 mg/kg used in Kim et al., 2009).

A.6.2. TCE Toxicokinetics in Rats: Liu et al. (2009)

Liu et al. (2009) measured TCE in blood of male rats after treatment with TCE by i.v. injection (0.1, 1.0, or 2.5 mg/kg) or aqueous gavage (0.0001, 0.001, 0.01, 0.1, 1, 2.5, 5, or 10 mg/kg). Almost all of the data from gavage exposures were within the inter-quartile region of the PBPK model population predictions, with all of it within the 95% confidence interval. For i.v. exposures, the data at 1 and 2.5 mg/kg were well simulated, but the time-course data at 0.1 mg/kg were substantially different in shape from that predicted by the PBPK model, with a lower initial concentration and longer half-life. The slower elimination rat at 0.1 mg/kg was noted by the study authors through use of noncompartamental analysis. There is no clear explanation for this discrepancy, particularly since the gavage data at this and even lower doses were well predicted by the PBPK model.

A.6.3. TCA Toxicokinetics in Mice and Rats: Mahle et al. (2001) and Green (2003a, b)

Three technical reports (Mahle et al., 2001; Green, 2003a, b) described by Sweeney et al. (2009) contained data on TCA toxicokinetics in mice and rats exposed to TCA in drinking water. These technical reports were provided to U.S. EPA by the Sweeney et al. (2009) authors.

TCA blood and liver concentrations were reported by Mahle et al. (2001) for male B6C3F1 mice and male Fischer 344 rats exposed to 0.1 g/L to 2 g/L TCA in drinking water for 3 or 14 days (12 to 270 mg/kg/d in mice and 7 to 150 mg/kg/d in rats). For mice, these data were all within the 95% confidence interval of PBPK model population predictions, with about half of these data within the interquartile region. For rats, all these data, except those for the 3-day exposure at 0.1 g/L, were within the 95% confidence interval of the PBPK model predictions. In addition, the median rat predictions were consistently higher than the data, although this could be explained by inter-study (strain, lot, etc.) variability.

TCA blood concentrations were reported by Green (2003a) for male and female B6C3F1 mice exposed to 0.5 g/L to 2.5 g/L TCA in drinking water for 5 days (130 to 600 mg/kg/d in males and 160 to 750 mg/kg/d in females). Notably, these animals consumed around twice as much water per day as compared to the mice reported by Mahle et al. (2001), and therefore received comparatively higher doses of TCA for the same TCE concentration in drinking water. In male mice, the data at the lower two doses (130 and 250 mg/kg/d) were within the inter-quartile region of the PBPK model predictions. The data for male mice at the highest dose (600 mg/kg/d) were below the inter-quartile region, but within the 95% confidence interval of the PBPK model predictions. In females, the data at the lower two doses (160 and 360 mg/kg/d) were mostly below the inter-quartile region, but within the 95% confidence interval of the PBPK model predictions, while about half the data at the highest dose were just below the 95% confidence interval.

TCA blood, plasma, and liver concentrations were reported by Green (2003b) for male PPARα-null mice, male 129/sv mice (the background strain of the PPARα-null mice), and male and female B6C3F1 mice, exposed to 1.0 g/L or 2.5 g/L TCA in drinking water for 5 days (male

B6C3F1 only) to 14 days.[2] In male PPARα-null mice, plasma and blood concentrations were within the inter-quartile region of the PBPK model predictions, while liver concentrations were below the inter-quartile region but within the 95% confidence interval. In male 129/sv mice, the plasma concentrations were within the inter-quartile region of the PBPK model predictions, while blood and liver concentrations were below the inter-quartile region but within the 95% confidence interval. In male B6C3F1 mice, all data were within the 95% confidence intervals of the PBPK model predictions, with about half within the inter-quartile region, and the rest above (plasma concentrations at the lower dose) or below (liver concentrations at all but the lowest dose at 5 days). In female B6C3F1 mice, plasma concentrations were below the inter-quartile region but within the 95% confidence region, while liver and blood concentrations were at or below the lower 95% confidence bound.

Overall, the predictions of the TCA submodel of the updated TCE PBPK model appear consistent with these data on the toxicokinetics of TCA after drinking water exposure in male rats and male mice. In female mice, the reported concentrations tends to be at the low end of or lower than those predicted by the PBPK model. Importantly, the data used for calibrating the mouse PBPK model parameters were predominantly in males, with only Fisher et al. (1991, 1993) reporting TCA plasma levels in female mice after TCE exposure. In addition, median PBPK model predictions at higher doses (>300 mg/kg/d), even in males, tended to be higher than the concentrations reported. While TCA kinetics after TCE exposure includes predicted internal production at these higher levels, previously published data on TCA kinetics alone only included doses up to 100 mg/kg, and only in males. Therefore, these results suggest that the median predictions of the TCA sub-model of the updated TCE PBPK model are somewhat less accurate for female mice and for higher doses of TCA (>300 mg/kg/d) in mice, though the 95% confidence intervals still cover the majority of the reported data. Finally, the ratio of blood to liver concentrations of ~1.4 reported in the mouse experiments in Mahle et al. (2001) were significantly different from the ratios of ~2.3 reported by Green (2003b), a difference for which there is no clear explanation given the similar experimental designs and common use the B6C3F1 mouse strain. Because median PBPK model predictions for the blood to liver concentration ratio for these studies are ~1.3, they are more consistent with the Mahle et al. (2001) data than with the Green (2003b) data.

Sweeney et al. (2009) also suggested that the available data, in conjunction with deterministic modeling using the TCA portion of the Hack et al. (2006) TCE PBPK model,

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[2] Sweeney et al. (2009) reported that blood concentrations in Green (2003b) were incorrect due to an arithmetic error owing to a change in chemical analytic methodology, and should have been multiplied by 2. This correction was included in the present analysis.

supported a hypothesis that the bioavailability of TCA in drinking water in mice is substantially less than 100%. Classically, oral bioavailability is assessed by comparing blood concentration profiles from oral and i.v. dosing experiments, because blood concentration data from oral dosing alone cannot distinguish fractional uptake from metabolism. Schultz et al. (1999) made this comparison in rats at a single dose of 82 mg/kg, and reported an empirical bioavailability of 116%, consistent with complete absorption. *A priori,* there would not seem to be a strong reason to suspect that oral absorption in mice would be significantly different from that in rats. As discussed above in the evaluation of Hack et al. (2006) model, available data strongly support clearance of TCA in addition to urinary excretion, based on the finding of less than 100% recovery in urine after i.v. dosing. In addition, as the current TCE PBPK model assumes 100% absorption for orally-administered TCA, and the PBPK model predictions are consistent with these data, it is likely that the limited bioavailability determined by Sweeney et al. (2009) was confounded by this additional clearance pathway unaccounted for by Hack et al. (2006). Therefore, the data are consistent with the combination of 100% absorption for orally-administered TCA and an additional clearance pathway for TCA other than urinary excretion in both rats and mice. This hypothesis could be further tested with additional experiments in mice directly comparing of TCA toxicokinetics (blood or plasma concentrations and urinary excretion) between i.v. and oral dosing.

A.7. UPDATED PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL CODE

The following pages contain the updated PBPK model code for the MCSim software (version 5.0.0). Additional details on baseline parameter derivations are included as inline documentation. Example simulation files containing prior distributions and experimental calibration data are available electronically:

- Mouse: Appendix.linked.files\TCE.1.2.3.3.Mouse.pop.example.in
- Rat: Appendix.linked.files\TCE.1.2.3.3.Rat.pop.example.in
- Human: Appendix.linked.files\TCE.1.2.3.3.Human.pop.example.in.

```
# TCE.risk.1.2.3.3.pop.model -- Updated TCE Risk Assessment Model
#
#### HISTORY OF HACK ET AL. (2006) MODEL
# Model code to correspond to the block diagram version of the model
# Edited by Deborah Keys to incorporate Lapare et al. 1995 data
# Last edited: August 6, 2004
# Translated into MCSim from acslXtreme CSL file by Eric Hack, started 31Aug2004
# Removed nonessential differential equations (i.e., AUCCBld) for MCMC runs.
# Changed QRap and QSlw calculations and added QTot to scale fractional flows
# back to 1 after sampling.
# Finished translating and verifying results on 15Sep2004.
# Changed QSlw calculation and removed QTot 21Sep2004.
# Removed diffusion-limited fat uptake 24Sep2004.
#### HISTORY OF U.S. EPA (2009) MODEL (CHIU ET AL., 2009)
# Extensively revised by U.S. EPA June 2007-June 2008
#         - Fixed hepatic plasma flow for TCA-submodel to include
#           portal vein (i.e., QGutLivPlas -- originally was just
#           QLivPlas, which was only hepatic artery).
#         - Clearer coding and in-line documentation
#         - Single model for 3 species
#         - Revised physiological parameters, with discussion of
#                   uncertainty and variability,
#         - In vitro data used for default metabolism parameters,
#                   with discussion of uncertainty and variability
#         - added TCE blood compartment
#         - added TCE kidney compartment, with GSH metabolism
#         - added DCVG compartment
#         - added additional outputs available from in vivo data
#         - removed DCA compartment
#         - added IA and PV dosing (for rats)
#         - Version 1.1 -- fixed urinary parameter scaling
#                             -- fixed VBod in kUrnTCOG (should be VBodTCOH)
#         - Version 1.1.1 -- changed some truncation limits (in commments only)
#         - Version 1.2 --
#                   -- removed TB compartment as currently coded
#                   -- added respiratory oxidative metabolism:
#                             3 states: AInhResp, AResp, AExhResp
#                   -- removed clearance from respiratory metabolism
#         - Version 1.2.1 -- changed oral dosing to be similar to IV
#         - Version 1.2.2 -- fixed default lung metabolism (additional
#                             scaling by lung/liver weight ratio)
#         - Version 1.2.3 -- fixed FracKidDCVC scaling
#         - Version 1.2.3.1 -- added output CDCVG_ND (no new dynamics)
#                   for non-detects of DCVG in blood
#         - Version 1.2.3.2 -- Exact version of non-detects likelihood
#         - Version 1.2.3.3 -- Error variances changed to "Ve_xxx"
#         NOTE -- lines with comment "(vrisk)" are used only for
#                   calculating dose metrics, and are commented out
#                   when doing MCMC runs.
#******************************************************************************
#***                  State Variable Specifications                        ***
#******************************************************************************
```

```
States = {
##-- TCE uptake
          AStom,              # Amount of TCE in stomach
          ADuod,              # oral gavage absorption -- mice and rats only
          AExc,               #(vrisk) excreted in feces from gavage (currently 0)
          AO,                 #(vrisk) total absorbed
          InhDose,  # Amount inhaled
##-- TCE in the body
          ARap,               # Amount in rapidly perfused tissues
          ASlw,               # Amount in slowly perfused tissues
          AFat,               # Amount in fat
          AGut,               # Amount in gut
          ALiv,               # Amount in liver
          AKid,               # Amount in Kidney -- previously in Rap tissue
          ABld,               # Amount in Blood -- previously in Rap tissue
          AInhResp, # Amount in respiratory lumen during inhalation
          AResp,              # Amount in respiratory tissue
          AExhResp, # Amount in respiratory lumen during exhalation
##-- TCA in the body
          AOTCA,              #(vrisk)
          AStomTCA, # Amount of TCA in stomach
          APlasTCA, # Amount of TCA in plasma #comment out for
          ABodTCA,  # Amount of TCA in lumped body compartment
          ALivTCA,  # Amount of TCA in liver
##-- TCA metabolized
          AUrnTCA,  # Cumulative Amount of TCA excreted in urine
          AUrnTCA_sat,        # Amount of TCA excreted that during times that had
                              # saturated measurements (for lower bounds)
          AUrnTCA_collect,# Cumulative Amount of TCA excreted in urine during
                              # collection times (for intermittent collection)
##-- TCOH in body
          AOTCOH,             #(vrisk)
          AStomTCOH,          # Amount of TCOH in stomach
          ABodTCOH,           # Amount of TCOH in lumped body compartment
          ALivTCOH,           # Amount of TCOH in liver
##-- TCOG in body
          ABodTCOG,           # Amount of TCOG in lumped body compartment
          ALivTCOG,           # Amount of TCOG in liver
          ABileTCOG,          # Amount of TCOG in bile (incl. gut)
          ARecircTCOG,        #(vrisk)
##-- TCOG excreted
          AUrnTCOG, # Amount of TCOG excreted in urine
          AUrnTCOG_sat,       # Amount of TCOG excreted that during times that had
                              # saturated measurements (for lower bounds)
          AUrnTCOG_collect,# Cumulative Amount of TCA excreted in urine during
                              # collection times (for intermittent collection)
##-- DCVG in body
          ADCVGIn,  #(vrisk)
          ADCVGmol, # Amount of DCVG in body in mmoles
          AMetDCVG, #(vrisk)
##-- DCVC in body
          ADCVCIn,  #(vrisk)
        ADCVC,                # Amount of DCVC in body
```

```
        ABioactDCVC,        #(vrisk)
##-- NAcDCVC excreted
        AUrnNDCVC,          # Amount of NAcDCVC excreted
##-- Other states for TCE
        ACh,                # Amount in closed chamber -- mice and rats only
        AExh,               # Amount exhaled
        AExhExp,  # Amount exhaled during expos  [to calc. retention]
##-- Metabolism
        AMetLiv1, #(vrisk) Amount metabolized by P450 in liver
        AMetLiv2, #(vrisk) Amount metabolized by GSH conjugation in liver
        AMetLng,  #(vrisk) Amount metabolized in the lung
        AMetKid,  #(vrisk)
        AMetTCOHTCA,        #(vrisk) Amount of TCOH metabolized to TCA
        AMetTCOHGluc,       #(vrisk) Amount of TCOH glucuronidated
        AMetTCOHOther,      #(vrisk)
        AMetTCA,  #(vrisk) Amount of TCA metabolized
##-- Other Dose metrics
        AUCCBld,  #(vrisk)
        AUCCLiv,  #(vrisk)
        AUCCKid,  #(vrisk)
        AUCCRap,  #(vrisk)
        AUCCTCOH, #(vrisk)
        AUCCBodTCOH,        #(vrisk)
        AUCTotCTCOH,        #(vrisk)
        AUCPlasTCAFree,     #(vrisk)
        AUCPlasTCA,         #(vrisk)
        AUCLivTCA,          #(vrisk)
        AUCCDCVG  #(vrisk)
};

#******************************************************************************
#***                 Input Variable Specifications                         ***
#******************************************************************************

Inputs = {
##-- TCE dosing
        Conc,               # Inhalation exposure conc. (ppm)
        IVDose,             # IV dose (mg/kg)
        PDose,              # Oral gavage dose (mg/kg)
        Drink,              # Drinking water dose (mg/kg/day)
        IADose,                       # Inter-arterial
        PVDose,                       # Portal Vein
##-- TCA dosing
        IVDoseTCA,          # IV dose (mg/kg) of TCA
        PODoseTCA,          # Oral dose (mg/kg) of TCA
##-- TCOH dosing
        IVDoseTCOH,         # IV dose (mg/kg) of TCOH
        PODoseTCOH,         # Oral dose (mg/kg) of TCOH
##-- Potentially time-varying parameters
        QPmeas,             # Measured value of Alveolar ventilation QP
        TCAUrnSat,          # Flag for saturated TCA urine
        TCOGUrnSat,         # Flag for saturated TCOG urine
        UrnMissing          # Flag for missing urine collection times
};

#******************************************************************************
#***                  Output Variable Specifications                        ***
#******************************************************************************
Outputs = {
#******************************************************************************
#*** Outputs for mass balance check
MassBalTCE,
TotDose,
TotTissue,
MassBalTCOH,
TotTCOHIn,
TotTCOHDose,
TotTissueTCOH,
TotMetabTCOH,
MassBalTCA,
TotTCAIn,
TotTissueTCA,
MassBalTCOG,
TotTCOGIn,
TotTissueTCOG,
MassBalDCVG,
MassBalDCVC,
AUrnNDCVCequiv,

#******************************************************************************
#*** Outputs that are potential dose metrics
        TotMetab, #(vrisk) Total metabolism
        TotMetabBW34, #(vrisk) Total metabolism/BW^3/4
        ATotMetLiv, #(vrisk) Total metabolism in liver
        AMetLiv1Liv, #(vrisk) Total oxidation in liver/liver volume
        AMetLivOther, #(vrisk) Total "other" oxidation in liver
        AMetLivOtherLiv, #(vrisk) Total "other" oxidation in liver/liver vol
        AMetLngResp, #(vrisk) oxiation in lung/respiratory tissue volume
        AMetGSH, #(vrisk) total GSH conjugation
        AMetGSHBW34, #(vrisk) total GSH conjugation/BW^3/4
        ABioactDCVCKid,     #(vrisk) Amount of DCVC bioactivated/kidney volume
# NEW
        TotDoseBW34, #(vrisk) mg intake / BW^3/4
        AMetLiv1BW34, #(vrisk) mg hepatic oxidative metabolism / BW^3/4
        TotOxMetabBW34, #(vrisk) mg oxidative metabolism / BW^3/4
        TotTCAInBW, #(vrisk) TCA production / BW
        AMetLngBW34, #(vrisk) oxiation in lung/BW^3/4
        ABioactDCVCBW34, #(vrisk) Amount of DCVC bioactivated/BW^3/4
        AMetLivOtherBW34, #(vrisk) Total "other" oxidation in liver/BW^3/4
#******************************************************************************
#*** Outputs for comparison to in vivo data
# TCE
RetDose,  # human - = (InhDose - AExhExp)
CAlv,     # needed for CAlvPPM
CAlvPPM,  # human
CInhPPM,  # mouse, rat
```

```
CInh,     # needed for CMixExh
CMixExh,  # rat - Mixed exhaled breath (mg/l)
CArt,     # rat, human - Arterial blood concentration
CVen,     # mouse, rat, human
CBldMix,  # rat - Concentration in mixed arterial+venous blood
                    # (used for cardiac puncture)
CFat,     # mouse, rat - Concentration in fat
CGut,     # rat
CRap,     # needed for unlumped tissues
CSlw,     # needed for unlumped tissues
CHrt,     # rat - Concentration in heart tissue [use CRap]
CKid,     # mouse, rat -  Concentration in kidney
CLiv,     # mouse, rat - Concentration in liver
CLung,    # mouse, rat - Concentration in lung [use CRap]
CMus,     # rat - Concentration in muscle [use CSlw]
CSpl,     # rat - Concentration in spleen [use CRap]
CBrn,     # rat - Concentration in brain [use CRap]
zAExh,    # mouse
zAExhpost,          # rat - Amount exhaled post-exposure (mg)

# TCOH
CTCOH,    # mouse, rat, human - TCOH concentration in blood
CKidTCOH, # mouse - TCOH concentration in kidney
CLivTCOH, # mouse - TCOH concentration in liver
CLungTCOH,          # mouse - TCOH concentration in lung

# TCA
CPlasTCA, # mouse, rat, human - TCA concentration in plasma
CBldTCA,  # mouse, rat, human - TCA concentration in blood
CBodTCA,  # needed for CKidTCA and CLungTCA
CKidTCA,  # mouse - TCA concentration in kidney
CLivTCA,  # mouse, rat - TCA concentration in liver
CLungTCA, # mouse - TCA concentration in lung
zAUrnTCA, # mouse, rat, human - Cumulative Urinary TCA
zAUrnTCA_collect,   # human - TCA measurements for intermittent collection
zAUrnTCA_sat,       # human - Saturated TCA measurements

# TCOG
zABileTCOG,         # rat - Amount of TCOG in bile (mg)
CTCOG,    # needed for CTCOGTCOH
CTCOGTCOH,          # mouse - TCOG concentration in blood (in TCOH-equiv)
CKidTCOGTCOH,       # mouse - TCOG concentration in kidney (in TCOH-equiv)
CLivTCOGTCOH,       # mouse - TCOG concentration in liver (in TCOH-equiv)
CLungTCOGTCOH,      # mouse - TCOG concentration in lung (in TCOH-equiv)
AUrnTCOGTCOH,       # mouse, rat, human - Cumulative Urinary TCOG (in TCOH-equiv)
AUrnTCOGTCOH_collect,         # human - TCOG (in TCOH-equiv) measurements for
                              # intermittent collection
AUrnTCOGTCOH_sat,   # human - Saturated TCOG (in TCOH-equiv) measurements

# Other
CDCVGmol,           # concentration of DCVG (mmol/l)
CDCVGmol0,          # Dummy variable without likelihood (for plotting)#(v1.2.3.1)
CDCVG_ND, # Non-detect of DCVG (<0.05 pmol/ml= 5e-5 mmol/l )#(v1.2.3.1)
                    # Output -ln(likelihood)#(v1.2.3.1)
zAUrnNDCVC,         # rat, human - Cumulative urinary NAcDCVC
AUrnTCTotMole,      # rat, human - Cumulative urinary TCOH+TCA in mmoles
TotCTCOH, # mouse, human - TCOH+TCOG Concentration (in TCOH-equiv)
TotCTCOHcomp,       # ONLY FOR COMPARISON WITH HACK
ATCOG,              # ONLY FOR COMPARISON WITH HACK
QPsamp,   # human - sampled value of alveolar ventilation rate

## PARAMETERS #(vrisk)

  QCnow, # (vrisk) #Cardiac output (L/hr)
  QP, # (vrisk) #Alveolar ventilation (L/hr)
  QFatCtmp, # (vrisk) #Scaled fat blood flow
  QGutCtmp, # (vrisk) #Scaled gut blood flow
  QLivCtmp, # (vrisk) #Scaled liver blood flow
  QSlwCtmp, # (vrisk) #Scaled slowly perfused blood flow
  QRapCtmp, # (vrisk) #Scaled rapidly perfused blood flow
  QKidCtmp, # (vrisk) #Scaled kidney blood flow
  DResp, # (vrisk) #Respiratory lumen:tissue diffusive clearance rate
  VFatCtmp, # (vrisk) #Fat fractional compartment volume
  VGutCtmp, # (vrisk) #Gut fractional compartment volume
  VLivCtmp, # (vrisk) #Liver fractional compartment volume
  VRapCtmp, # (vrisk) #Rapidly perfused fractional compartment volume
  VRespLumCtmp, # (vrisk) # Fractional volume of respiratory lumen
  VRespEffCtmp, # (vrisk) #Effective fractional volume of respiratory tissue
  VKidCtmp, # (vrisk) #Kidney fractional compartment volume
  VBldCtmp, # (vrisk) #Blood fractional compartment volume
  VSlwCtmp, # (vrisk) #Slowly perfused fractional compartment volume
  VPlasCtmp, # (vrisk) #Plasma fractional compartment volume
  VBodCtmp, # (vrisk) #TCA Body fractional compartment volume [not incl.
blood+liver]
  VBodTCOHCtmp, # (vrisk) #TCOH/G Body fractional compartment volume [not incl.
liver]
  PB, # (vrisk) #TCE Blood/air partition coefficient
  PFat, # (vrisk) #TCE Fat/Blood partition coefficient
  PGut, # (vrisk) #TCE Gut/Blood partition coefficient
  PLiv, # (vrisk) #TCE Liver/Blood partition coefficient
  PRap, # (vrisk) #TCE Rapidly perfused/Blood partition coefficient
  PResp, # (vrisk) #TCE Respiratory tissue:air partition coefficient
  PKid, # (vrisk) #TCE Kidney/Blood partition coefficient
  PSlw, # (vrisk) #TCE Slowly perfused/Blood partition coefficient
  TCAPlas, # (vrisk) #TCA blood/plasma concentration ratio
  PBodTCA, # (vrisk) #Free TCA Body/blood plasma partition coefficient
  PLivTCA, # (vrisk) #Free TCA Liver/blood plasma partition coefficient
  kDissoc, # (vrisk) #Protein/TCA dissociation constant (umole/L)
  BMax, # (vrisk) #Maximum binding concentration (umole/L)
  PBodTCOH, # (vrisk) #TCOH body/blood partition coefficient
  PLivTCOH, # (vrisk) #TCOH liver/body partition coefficient
  PBodTCOG, # (vrisk) #TCOG body/blood partition coefficient
  PLivTCOG, # (vrisk) #TCOG liver/body partition coefficient
  VDCVG, # (vrisk) #DCVG effective volume of distribution
  kAS, # (vrisk) #TCE Stomach absorption coefficient (/hr)
  kTSD, # (vrisk) #TCE Stomach-duodenum transfer coefficient (/hr)
```

```
  kAD, # (vrisk) #TCE Duodenum absorption coefficient (/hr)
  kTD, # (vrisk) #TCE Duodenum-feces transfer coefficient (/hr)
  kASTCA, # (vrisk) #TCA Stomach absorption coefficient (/hr)
  kASTCOH, # (vrisk) #TCOH Stomach absorption coefficient (/hr)
  VMax, # (vrisk) #VMax for hepatic TCE oxidation (mg/hr)
  KM, # (vrisk) #KM for hepatic TCE oxidation (mg/L)
  FracOther, # (vrisk) #Fraction of hepatic TCE oxidation not to TCA+TCOH
  FracTCA, # (vrisk) #Fraction of hepatic TCE oxidation to TCA
  VMaxDCVG, # (vrisk) #VMax for hepatic TCE GSH conjugation (mg/hr)
  KMDCVG, # (vrisk) #KM for hepatic TCE GSH conjugation (mg/L)
  VMaxKidDCVG, # (vrisk) #VMax for renal TCE GSH conjugation (mg/hr)
  KMKidDCVG, # (vrisk) #KM for renal TCE GSH conjugation (mg/L)
  FracKidDCVC, # (vrisk) #Fraction of renal TCE GSH conj. "directly" to DCVC
              # (vrisk) #(i.e., via first pass)
  VMaxClara, # (vrisk) #VMax for Tracheo-bronchial TCE oxidation (mg/hr)
  KMClara, # (vrisk) #KM for Tracheo-bronchial TCE oxidation (mg/L)
  FracLungSys, # (vrisk) #Fraction of respiratory metabolism to systemic circ.
  VMaxTCOH, # (vrisk) #VMax for hepatic TCOH->TCA (mg/hr)
  KMTCOH, # (vrisk) #KM for hepatic TCOH->TCA (mg/L)
  VMaxGluc, # (vrisk) #VMax for hepatic TCOH->TCOG (mg/hr)
  KMGluc, # (vrisk) #KM for hepatic TCOH->TCOG (mg/L)
  kMetTCOH, # (vrisk) #Rate constant for hepatic TCOH->other (/hr)
  kUrnTCA, # (vrisk) #Rate constant for TCA plasma->urine (/hr)
  kMetTCA, # (vrisk) #Rate constant for hepatic TCA->other (/hr)
  kBile, # (vrisk) #Rate constant for TCOG liver->bile (/hr)
  kEHR, # (vrisk) #Lumped rate constant for TCOG bile->TCOH liver (/hr)
  kUrnTCOG, # (vrisk) #Rate constant for TCOG->urine (/hr)
  kDCVG, # (vrisk) #Rate constant for hepatic DCVG->DCVC (/hr)
  kNAT, # (vrisk) #Lumped rate constant for DCVC->Urinary NAcDCVC (/hr)
  kKidBioact, # (vrisk) #Rate constant for DCVC bioactivation (/hr)

## Misc
  RUrnTCA, #(vrisk)
  RUrnTCOGTCOH, #(vrisk)
  RUrnNDCVC, #(vrisk)
  RAO,
  CVenMole,
  CPlasTCAMole,
  CPlasTCAFreeMole
};

#*****************************************************************************
#***                  Global Constants                                     ***
#*****************************************************************************

# Molecular Weights
        MWTCE = 131.39;        # TCE
        MWDCA = 129.0;        # DCA
       MWDCVC = 216.1;        # DCVC
        MWTCA = 163.5;        # TCA
      MWChlor = 147.5;        # Chloral
       MWTCOH = 149.5;        # TCOH
   MWTCOHGluc = 325.53;       # TCOH-Gluc
     MWNADCVC = 258.8;        # N Acetyl DCVC

# Stoichiometry
 StochChlorTCE = MWChlor / MWTCE;
   StochTCATCE = MWTCA / MWTCE;
  StochTCATCOH = MWTCA / MWTCOH;
  StochTCOHTCE = MWTCOH / MWTCE;
 StochGlucTCOH = MWTCOHGluc / MWTCOH;
 StochTCOHGluc = MWTCOH / MWTCOHGluc;
  StochTCEGluc = MWTCE / MWTCOHGluc;
  StochDCVCTCE = MWDCVC / MWTCE;
        StochN = MWNADCVC / MWDCVC;
   StochDCATCE = MWDCA / MWTCE;

#*****************************************************************************
#***                  Global Model Parameters                              ***
#*****************************************************************************
# These are the actual model parameters used in "dynamics."
# Values that are assigned in the "initialize" section,
# are all set to 1 to avoid confusion.

#*****************************************************************************
# Flows
QC         = 1;      # Cardiac output (L/hr)
QPsamp     = 1;      # Alveolar ventilation (L/hr)
VPR        = 1;      # Alveolar ventilation-perfusion ratio
QFatCtmp   = 1;      # Scaled fat blood flow
QGutCtmp   = 1;      # Scaled gut blood flow
QLivCtmp   = 1;      # Scaled liver blood flow
QSlwCtmp   = 1;      # Scaled slowly perfused blood flow
DResptmp   = 1;      # Respiratory lumen:tissue diffusive clearance rate (L/hr)
[scaled to QP]
QKidCtmp   = 1;      # Scaled kidney blood flow
FracPlas   = 1;      # Fraction of blood that is plasma (1-hematocrit)
#*****************************************************************************
# Volumes
VFat       = 1;      # Fat compartment volume (L)
VGut       = 1;      # Gut compartment volume (L)
VLiv       = 1;      # Liver compartment volume (L)
VRap       = 1;      # Rapidly perfused compartment volume (L)
VRespLum   = 1;      # Volume of respiratory lumen (L air)
VRespEfftmp          = 1;      #(vrisk) volume for respiratory tissue (L)
VRespEff   = 1;      # Effective volume for respiratory tissue (L air) = V(tissue) *
Resp:Air partition coefficient
VKid       = 1;      # Kidney compartment volume (L)
VBld       = 1;      # Blood compartment volume (L)
VSlw       = 1;      # Slowly perfused compartment volume (L)
VPlas      = 1;      # Plasma compartment volume [fraction of blood] (L)
VBod       = 1;      # TCA Body compartment volume [not incl. blood+liver] (L)
VBodTCOH   = 1;      # TCOH/G Body compartment volume [not incl. liver] (L)
#*****************************************************************************
# Distribution/partitioning
PB         = 1;      # TCE Blood/air partition coefficient
```

```
PFat      = 1;      # TCE Fat/Blood partition coefficient
PGut      = 1;      # TCE Gut/Blood partition coefficient
PLiv      = 1;      # TCE Liver/Blood partition coefficient
PRap      = 1;      # TCE Rapidly perfused/Blood partition coefficient
PResp     = 1;      # TCE Respiratory tissue:air partition coefficient
PKid      = 1;      # TCE Kidney/Blood partition coefficient
PSlw      = 1;      # TCE Slowly perfused/Blood partition coefficient
TCAPlas   = 1;      # TCA blood/plasma concentration ratio
PBodTCA   = 1;      # Free TCA Body/blood plasma partition coefficient
PLivTCA   = 1;      # Free TCA Liver/blood plasma partition coefficient
kDissoc   = 1;      # Protein/TCA dissociation constant (umole/L)
BMax      = 1;      # Protein concentration (UNITS?)
PBodTCOH  = 1;      # TCOH body/blood partition coefficient
PLivTCOH  = 1;      # TCOH liver/body partition coefficient
PBodTCOG  = 1;      # TCOG body/blood partition coefficient
PLivTCOG  = 1;      # TCOG liver/body partition coefficient
VDCVG     = 1;      # DCVG effective volume of distribution
#*****************************************************************************
# Oral absorption
kTSD      = 1.4;    # TCE Stomach-duodenum transfer coefficient (/hr)
kAS       = 1.4;    # TCE Stomach absorption coefficient (/hr)
kTD       = 0.1;    # TCE Duodenum-feces transfer coefficient (/hr)
kAD       = 0.75;   # TCE Duodenum absorption coefficient (/hr)
kASTCA    = 0.75;   # TCA Stomach absorption coefficient (/hr)
kASTCOH   = 0.75;   # TCOH Stomach absorption coefficient (/hr)
#*****************************************************************************
# TCE Metabolism
VMax      = 1;      # VMax for hepatic TCE oxidation (mg/hr)
KM        = 1;      # KM for hepatic TCE oxidation (mg/L)
FracOther = 1;      # Fraction of hepatic TCE oxidation not to TCA+TCOH
FracTCA   = 1;      # Fraction of hepatic TCE oxidation to TCA
VMaxDCVG  = 1;      # VMax for hepatic TCE GSH conjugation (mg/hr)
KMDCVG    = 1;      # KM for hepatic TCE GSH conjugation (mg/L)
VMaxKidDCVG         = 1;      # VMax for renal TCE GSH conjugation (mg/hr)
KMKidDCVG = 1;      # KM for renal TCE GSH conjugation (mg/L)
VMaxClara = 1;      # VMax for Tracheo-bronchial TCE oxidation (mg/hr)
KMClara   = 1;      # KM for Tracheo-bronchial TCE oxidation (mg/L)
                    # but in units of air concentration
FracLungSys         = 1;      # Fraction of respiratory oxidative metabolism that
enters systemic circulation

#*****************************************************************************
# TCOH metabolism
VMaxTCOH  = 1;      # VMax for hepatic TCOH->TCA (mg/hr)
KMTCOH    = 1;      # KM for hepatic TCOH->TCA (mg/L)
VMaxGluc  = 1;      # VMax for hepatic TCOH->TCOG (mg/hr)
KMGluc    = 1;      # KM for hepatic TCOH->TCOG (mg/L)
kMetTCOH  = 1;      # Rate constant for hepatic TCOH->other (/hr)
#*****************************************************************************
# TCA metabolism/clearance
kUrnTCA   = 1;      # Rate constant for TCA plasma->urine (/hr)
kMetTCA   = 1;      # Rate constant for hepatic TCA->other (/hr)
#*****************************************************************************
```

```
# TCOG metabolism/clearance
kBile     = 1;      # Rate constant for TCOG liver->bile (/hr)
kEHR      = 1;      # Lumped rate constant for TCOG bile->TCOH liver (/hr)
kUrnTCOG  = 1;      # Rate constant for TCOG->urine (/hr)
#*****************************************************************************
# DCVG metabolism
kDCVG     = 1;      # Rate constant for hepatic DCVG->DCVC (/hr)
FracKidDCVC         = 1;      # Fraction of renal TCE GSH conj. "directly" to DCVC
(i.e., via first pass)
#*****************************************************************************
# DCVC metabolism/clearance
kNAT      = 1;      # Lumped rate constant for DCVC->Urinary NAcDCVC (/hr)
kKidBioact          = 1;      # Rate constant for DCVC bioactivation (/hr)
#*****************************************************************************
# Closed chamber and other exposure parameters
Rodents   = 1;      # Number of rodents in closed chamber data
VCh       = 1;      # Chamber volume for closed chamber data
kLoss     = 1;      # Rate constant for closed chamber air loss
CC        = 0.0;    # Initial chamber concentration (ppm)
TChng     = 0.003;  # IV infusion duration (hour)
#*****************************************************************************
## Flag for species, sex -- these are global parameters
BW        = 0.0;  # Species-specific defaults during initialization
BW75      = 0.0;    #(vrisk) Variable for BW^3/4
Male      = 1.0;    # 1 = male, 0 = female
Species   = 1.0;    # 1 = human, 2 = rat, 3 = mouse

#*****************************************************************************
#***                Potentially measured covariates (constants)           ***
#*****************************************************************************
BWmeas    = 0.0;    # Body weight
VFatCmeas = 0.0; # Fractional volume fat
PBmeas    = 0.0;    # Measured blood-air partition coefficient
Hematocritmeas = 0.0; # Measured hematocrit -- used for FracPlas = 1 - HCt
CDCVGmolLD = 5e-5; # Detection limit of CDCVGmol#(v1.2.3.1)

#*****************************************************************************
#***                Global Sampling Parameters                              ***
#*****************************************************************************
# These parameters are potentially sampled/calibrated in the MCMC or MC
# analyses.  The default values here are used if no sampled value is given.
# M_ indicates population mean parameters used only in MC sampling
# V_ indicates a population variance parameter used in MC and MCMC sampling

# Flow Rates
lnQCC     = 0.0;    # Scaled by BW^0.75 and species-specific central estimates
lnVPRC    = 0.0;    # Scaled to species-specific central estimates

# Fractional Blood Flows to Tissues (fraction of cardiac output)
QFatC     = 1.0;    # Scaled to species-specific central estimates
QGutC     = 1.0;    # Scaled to species-specific central estimates
QLivC     = 1.0;    # Scaled to species-specific central estimates
QSlwC     = 1.0;    # Scaled to species-specific central estimates
```

```
QKidC     = 1.0;    # Scaled to species-specific central estimates
FracPlasC = 1.0;    # Scaled to species-specific central estimates
lnDRespC  = 0.0;    # Scaled to alveolar ventilation rate in dynamics

# Fractional Tissue Volumes (fraction of BW)
VFatC     = 1.0;    # Scaled to species-specific central estimates
VGutC     = 1.0;    # Scaled to species-specific central estimates
VLivC     = 1.0;    # Scaled to species-specific central estimates
VRapC     = 1.0;    # Scaled to species-specific central estimates
VRespLumC = 1.0;    # Scaled to species-specific central estimates
VRespEffC = 1.0;    # Scaled to species-specific central estimates

VKidC     = 1.0;    # Scaled to species-specific central estimates
VBldC     = 1.0;    # Scaled to species-specific central estimate

# Partition Coefficients for TCE
lnPBC     = 0.0;    # Scaled to species-specific central estimates
lnPFatC   = 0.0;    # Scaled to species-specific central estimates
lnPGutC   = 0.0;    # Scaled to species-specific central estimates
lnPLivC   = 0.0;    # Scaled to species-specific central estimates
lnPRapC   = 0.0;    # Scaled to species-specific central estimates
lnPRespC  = 0.0;    # Scaled to species-specific central estimates
lnPKidC   = 0.0;    # Scaled to species-specific central estimates
lnPSlwC   = 0.0;    # Scaled to species-specific central estimates

# Partition Coefficients for TCA
lnPRBCPlasTCAC      = 0.0;    # Scaled to species-specific central estimates
lnPBodTCAC          = 0.0;    # Scaled to species-specific central estimates
lnPLivTCAC          = 0.0;    # Scaled to species-specific central estimates

# Plasma Binding for TCA
lnkDissocC          = 0.0;    # Scaled to species-specific central estimates
lnBMaxkDC = 0.0;    # Scaled to species-specific central estimates

# Partition Coefficients for TCOH and TCOG
lnPBodTCOHC         = 0.0;    # Scaled to species-specific central estimates
lnPLivTCOHC         = 0.0;    # Scaled to species-specific central estimates
lnPBodTCOGC         = 0.0;    # Scaled to species-specific central estimates
lnPLivTCOGC         = 0.0;    # Scaled to species-specific central estimates
lnPeffDCVG          = 0.0;    # Scaled to species-specific central estimates

# Oral Absorption rates
lnkTSD    = 0.336;
lnkAS     = 0.336;
lnkTD     = -2.303;
lnkAD     = -0.288;
lnkASTCA  = -0.288;
lnkASTCOH = -0.288;

# TCE Metabolism
lnVMaxC   = 0.0;    # Scaled by liver weight and species-specific central estimates
lnKMC     = 0.0;    # Scaled to species-specific central estimates
lnClC     = 0.0;    # Scaled to species-specific central estimates
lnFracOtherC        = 0.0;    # Ratio of DCA to non-DCA
lnFracTCAC          = 0.0;    # Ratio of TCA to TCOH
lnVMaxDCVGC         = 0.0;    # Scaled by liver weight and species-specific central
estimates
lnClDCVGC = 0.0;    # Scaled to species-specific central estimates
lnKMDCVGC = 0.0;    # Scaled to species-specific central estimates
lnVMaxKidDCVGC      = 0.0;    # Scaled by kidney weight and species-specific central
estimates
lnClKidDCVGC        = 0.0;    # Scaled to species-specific central estimates
lnKMKidDCVGC        = 0.0;    # Scaled to species-specific central estimates
lnVMaxLungLivC      = 0.0;    # Ratio of lung Vmax to liver Vmax,
                              # Scaled to species-specific central estimates
lnKMClara = 0.0;    # now in units of air concentration

# Clearance in lung
lnFracLungSysC      = 0.0;    # ratio of systemic to local clearance of lung
oxidation

# TCOH Metabolism
lnVMaxTCOHC         = 0.0;    # Scaled by BW^0.75
lnClTCOHC = 0.0;    # Scaled by BW^0.75
lnKMTCOH  = 0.0;    #
lnVMaxGlucC         = 0.0;    # Scaled by BW^0.75
lnClGlucC = 0.0;    # Scaled by BW^0.75
lnKMGluc  = 0.0;    #
lnkMetTCOHC         = 0.0;    # Scaled by BW^-0.25

# TCA Metabolism/clearance
lnkUrnTCAC          = 0.0;    # Scaled by (plasma volume)^-1 and species-specific
central estimates
lnkMetTCAC          = 0.0;    # Scaled by BW^-0.25

# TCOG excretion and reabsorption
lnkBileC  = 0.0;    # Scaled by BW^-0.25
lnkEHRC   = 0.0;    # Scaled by BW^-0.25
lnkUrnTCOGC         = 0.0;    # Scaled by (blood volume)^-1 and species-specific
central estimates

# DCVG metabolism
lnFracKidDCVCC      = 0.0;    # Ratio of "directly" to DCVC to systemic DCVG
lnkDCVGC  = 0.0;    # Scaled by BW^-0.25

# DCVC metabolism
lnkNATC   = 0.0;    # Scaled by BW^-0.25
lnkKidBioactC       = 0.0;    # Scaled by BW^-0.25

# Closed chamber parameters
NRodents  = 1;      #
VChC      = 1;      #
lnkLossC  = 0;      #

#******************************************************************************
# Population means
```

```
#
# These are given truncated normal or uniform distributions, depending on
#         what prior information is available.  Note that these distributions
#         reflect uncertainty in the population mean, not inter-individual
#         variability.  Normal distributions are truncated at 2, 3, or 4 SD.
#                   For fractional volumes and flows, 2xSD
#                   For plasma fraction, 3xSD
#                   For cardiac output and ventilation-perfusion ratio, 4xSD
#                   For all others, 3xSD
#         For uniform distributions, range of 1e2 to 1e8 fold, centered on
#                   central estimate.
#
M_lnQCC   = 1.0;
M_lnVPRC  = 1.0;
M_QFatC   = 1.0;
M_QGutC   = 1.0;
M_QLivC   = 1.0;
M_QSlwC   = 1.0;
M_QKidC   = 1.0;
M_FracPlasC         = 1.0;
M_lnDRespC = 1.0;
M_VFatC   = 1.0;
M_VGutC   = 1.0;
M_VLivC   = 1.0;
M_VRapC   = 1.0;
M_VRespLumC = 1.0;
M_VRespEffC = 1.0;
M_VKidC   = 1.0;
M_VBldC   = 1.0;
M_lnPBC   = 1.0;
M_lnPFatC = 1.0;
M_lnPGutC = 1.0;
M_lnPLivC = 1.0;
M_lnPRapC = 1.0;
M_lnPRespC          = 1.0;
M_lnPKidC = 1.0;
M_lnPSlwC = 1.0;
M_lnPRBCPlasTCAC    = 1.0;
M_lnPBodTCAC        = 1.0;
M_lnPLivTCAC        = 1.0;
M_lnkDissocC        = 1.0;
M_lnBMaxkDC         = 1.0;
M_lnPBodTCOHC       = 1.0;
M_lnPLivTCOHC       = 1.0;
M_lnPBodTCOGC       = 1.0;
M_lnPLivTCOGC       = 1.0;
M_lnPeffDCVG        = 1.0;
M_lnkTSD  = 1.0;
M_lnkAS   = 1.0;
M_lnkTD   = 1.0;
M_lnkAD   = 1.0;
M_lnkASTCA          = 1.0;
M_lnkASTCOH         = 1.0;
M_lnVMaxC = 1.0;
M_lnKMC   = 1.0;
M_lnClC   = 1.0;
M_lnFracOtherC      = 1.0;
M_lnFracTCAC        = 1.0;
M_lnVMaxDCVGC       = 1.0;
M_lnClDCVGC         = 1.0;
M_lnKMDCVGC         = 1.0;
M_lnVMaxKidDCVGC    = 1.0;
M_lnClKidDCVGC      = 1.0;
M_lnKMKidDCVGC      = 1.0;
M_lnVMaxLungLivC    = 1.0;
M_lnKMClara         = 1.0;
M_lnFracLungSysC    = 1.0;
M_lnVMaxTCOHC       = 1.0;
M_lnClTCOHC         = 1.0;
M_lnKMTCOH          = 1.0;
M_lnVMaxGlucC       = 1.0;
M_lnClGlucC         = 1.0;
M_lnKMGluc          = 1.0;
M_lnkMetTCOHC       = 1.0;
M_lnkUrnTCAC        = 1.0;
M_lnkMetTCAC        = 1.0;
M_lnkBileC          = 1.0;
M_lnkEHRC = 1.0;
M_lnkUrnTCOGC       = 1.0;
M_lnFracKidDCVCC    = 1.0;
M_lnkDCVGC          = 1.0;
M_lnkNATC = 1.0;
M_lnkKidBioactC     = 1.0;


#******************************************************************************
# Population Variances
#
# These are given InvGamma(alpha,beta) distributions.  The parameterization
#         for alpha and beta is given by:
#                   alpha = (n-1)/2
#                   beta = s^2*(n-1)/2
#         where n = number of data points, and s^2 is the sample variance
#         Sum(x_i^2)/n - <x>^2.
# Generally, for parameters for which there is no direct data, assume a
#         value of n = 5 (alpha = 2).  For a sample variance s^2, this gives
#         an expected value for the standard deviation <sigma> = 0.9*s,
#         a median [2.5%,97.5%] of 1.1*s [0.6*s,2.9*s].
#
V_lnQCC   = 1.0;
V_lnVPRC  = 1.0;
V_QFatC   = 1.0;
V_QGutC   = 1.0;
V_QLivC   = 1.0;
V_QSlwC   = 1.0;
V_QKidC   = 1.0;
```

```
V_FracPlasC         = 1.0;
V_lnDRespC = 1.0;
V_VFatC   = 1.0;
V_VGutC   = 1.0;
V_VLivC   = 1.0;
V_VRapC   = 1.0;
V_VRespLumC = 1.0;
V_VRespEffC = 1.0;
V_VKidC   = 1.0;
V_VBldC   = 1.0;
V_lnPBC   = 1.0;
V_lnPFatC = 1.0;
V_lnPGutC = 1.0;
V_lnPLivC = 1.0;
V_lnPRapC = 1.0;
V_lnPRespC          = 1.0;
V_lnPKidC = 1.0;
V_lnPSlwC = 1.0;
V_lnPRBCPlasTCAC    = 1.0;
V_lnPBodTCAC        = 1.0;
V_lnPLivTCAC        = 1.0;
V_lnkDissocC        = 1.0;
V_lnBMaxkDC         = 1.0;
V_lnPBodTCOHC       = 1.0;
V_lnPLivTCOHC       = 1.0;
V_lnPBodTCOGC       = 1.0;
V_lnPLivTCOGC       = 1.0;
V_lnPeffDCVG        = 1.0;
V_lnkTSD  = 1.0;
V_lnkAS   = 1.0;
V_lnkTD   = 1.0;
V_lnkAD   = 1.0;
V_lnkASTCA          = 1.0;
V_lnkASTCOH         = 1.0;
V_lnVMaxC = 1.0;
V_lnKMC   = 1.0;
V_lnClC   = 1.0;
V_lnFracOtherC      = 1.0;
V_lnFracTCAC        = 1.0;
V_lnVMaxDCVGC       = 1.0;
V_lnClDCVGC         = 1.0;
V_lnKMDCVGC         = 1.0;
V_lnVMaxKidDCVGC    = 1.0;
V_lnClKidDCVGC      = 1.0;
V_lnKMKidDCVGC      = 1.0;
V_lnVMaxLungLivC    = 1.0;
V_lnKMClara         = 1.0;
V_lnFracLungSysC    = 1.0;
V_lnVMaxTCOHC       = 1.0;
V_lnClTCOHC         = 1.0;
V_lnKMTCOH          = 1.0;
V_lnVMaxGlucC       = 1.0;
V_lnClGlucC         = 1.0;
V_lnKMGluc          = 1.0;
V_lnkMetTCOHC       = 1.0;
V_lnkUrnTCAC        = 1.0;
V_lnkMetTCAC        = 1.0;
V_lnkBileC          = 1.0;
V_lnkEHRC = 1.0;
V_lnkUrnTCOGC       = 1.0;
V_lnFracKidDCVCC    = 1.0;
V_lnkDCVGC          = 1.0;
V_lnkNATC = 1.0;
V_lnkKidBioactC     = 1.0;

#******************************************************************************
# Measurement error variances for output

Ve_RetDose          = 1;
Ve_CAlv   = 1;
Ve_CAlvPPM          = 1;
Ve_CInhPPM          = 1;
Ve_CInh   = 1;
Ve_CMixExh          = 1;
Ve_CArt   = 1;
Ve_CVen   = 1;
Ve_CBldMix          = 1;

Ve_CFat   = 1;
Ve_CGut   = 1;
Ve_CRap   = 1;
Ve_CSlw   = 1;
Ve_CHrt   = 1;
Ve_CKid   = 1;
Ve_CLiv   = 1;
Ve_CLung  = 1;
Ve_CMus   = 1;
Ve_CSpl   = 1;
Ve_CBrn   = 1;
Ve_zAExh  = 1;
Ve_zAExhpost        = 1;


Ve_CTCOH  = 1;
Ve_CKidTCOH         = 1;
Ve_CLivTCOH         = 1;
Ve_CLungTCOH        = 1;


Ve_CPlasTCA         = 1;
Ve_CBldTCA          = 1;
Ve_CBodTCA          = 1;
Ve_CKidTCA          = 1;
Ve_CLivTCA          = 1;
Ve_CLungTCA         = 1;
Ve_zAUrnTCA         = 1;
```

```
Ve_zAUrnTCA_collect = 1;
Ve_zAUrnTCA_sat     = 1;


Ve_zABileTCOG       = 1;
Ve_CTCOG  = 1;
Ve_CTCOGTCOH        = 1;
Ve_CKidTCOGTCOH     = 1;
Ve_CLivTCOGTCOH     = 1;
Ve_CLungTCOGTCOH    = 1;
Ve_AUrnTCOGTCOH     = 1;
Ve_AUrnTCOGTCOH_collect       = 1;

Ve_AUrnTCOGTCOH_sat = 1;


Ve_CDCVGmol         = 1;
Ve_zAUrnNDCVC       = 1;
Ve_AUrnTCTotMole    = 1;
Ve_TotCTCOH         = 1;
Ve_QPsamp = 1;

#*******************************************************************************
#***                  Defaults for input parameters                          ***
#*******************************************************************************
##-- TCE dosing
          Conc = 0.0;         # Inhalation exposure conc. (ppm)
          IVDose = 0.0;       # IV dose (mg/kg)
          PDose = 0.0;        # Oral gavage dose (mg/kg)
          Drink = 0.0;        # Drinking water dose (mg/kg/day)
          IADose = 0.0;       # Intraarterial dose (mg/kg)
          PVDose = 0.0;       # Portal vein dose (mg/kg)
##-- TCA dosing
          IVDoseTCA = 0.0;# IV dose (mg/kg) of TCA
          PODoseTCA = 0.0;# Oral dose (mg/kg) of TCA
##-- TCOH dosing
          IVDoseTCOH = 0.0;# IV dose (mg/kg) of TCOH
          PODoseTCOH = 0.0;# Oral dose (mg/kg) of TCOH
##-- Potentially time-varying parameters
          QPmeas = 0.0;       # Measured value of Alveolar ventilation QP
          TCAUrnSat = 0.0;# Flag for saturated TCA urine
          TCOGUrnSat = 0.0;# Flag for saturated TCOG urine
          UrnMissing = 0.0;# Flag for missing urine collection times

Initialize {

#*******************************************************************************
#***                  Parameter Initialization and Scaling                   ***
#*******************************************************************************
# Model Parameters (used in dynamics):
#         QC                  Cardiac output (L/hr)
#         VPR                 Ventilation-perfusion ratio
#         QPsamp              Alveolar ventilation (L/hr)
#         QFatCtmp  Scaled fat blood flow
#         QGutCtmp  Scaled gut blood flow
#         QLivCtmp  Scaled liver blood flow
#         QSlwCtmp  Scaled slowly perfused blood flow
#         DResptmp  Respiratory lumen:tissue diffusive clearance rate
#         QKidCtmp  Scaled kidney blood flow
#         FracPlas  Fraction of blood that is plasma (1-hematocrit)
#         VFat                Fat compartment volume (L)
#         VGut                Gut compartment volume (L)
#         VLiv                Liver compartment volume (L)
#         VRap                Rapidly perfused compartment volume (L)
#         VRespLum  Volume of respiratory lumen (L air)
#         VRespEff  Effective volume of respiratory tissue (L air)
#         VKid                Kidney compartment volume (L)
#         VBld                Blood compartment volume (L)
#         VSlw                Slowly perfused compartment volume (L)
#         VPlas               Plasma compartment volume [fraction of blood] (L)
#         VBod                TCA Body compartment volume [not incl. blood+liver]
(L)
#         VBodTCOH  TCOH/G Body compartment volume [not incl. liver] (L)
#         PB                  TCE Blood/air partition coefficient
#         PFat                TCE Fat/Blood partition coefficient
#         PGut                TCE Gut/Blood partition coefficient
#         PLiv                TCE Liver/Blood partition coefficient
#         PRap                TCE Rapidly perfused/Blood partition coefficient
#         PResp               TCE Respiratory tissue:air partition coefficient
#         PKid                TCE Kidney/Blood partition coefficient
#         PSlw                TCE Slowly perfused/Blood partition coefficient
#         TCAPlas             TCA blood/plasma concentration ratio
#         PBodTCA             Free TCA Body/blood plasma partition coefficient
#         PLivTCA             Free TCA Liver/blood plasma partition coefficient
#         kDissoc             Protein/TCA dissociation constant (umole/L)
#         BMax                Maximum binding concentration (umole/L)
#         PBodTCOH  TCOH body/blood partition coefficient
#         PLivTCOH  TCOH liver/body partition coefficient
#         PBodTCOG  TCOG body/blood partition coefficient
#         PLivTCOG  TCOG liver/body partition coefficient
#         kAS                 TCE Stomach absorption coefficient (/hr)
#         kTSD                TCE Stomach-duodenum transfer coefficient (/hr)
#         kAD                 TCE Duodenum absorption coefficient (/hr)
#         kTD                 TCE Duodenum-feces transfer coefficient (/hr)
#         kASTCA              TCA Stomach absorption coefficient (/hr)
#         kASTCOH             TCOH Stomach absorption coefficient (/hr)
#         VMax                VMax for hepatic TCE oxidation (mg/hr)
#         KM                  KM for hepatic TCE oxidation (mg/L)
#         FracOther Fraction of hepatic TCE oxidation not to TCA+TCOH
#         FracTCA             Fraction of hepatic TCE oxidation to TCA
#         VMaxDCVG  VMax for hepatic TCE GSH conjugation (mg/hr)
#         KMDCVG              KM for hepatic TCE GSH conjugation (mg/L)
#         VMaxKidDCVG         VMax for renal TCE GSH conjugation (mg/hr)
#         KMKidDCVG KM for renal TCE GSH conjugation (mg/L)
#         VMaxClara VMax for Tracheo-bronchial TCE oxidation (mg/hr)
#         KMClara             KM for Tracheo-bronchial TCE oxidation (mg/L)
```

```
#         FracLungSys         Fraction of respiratory metabolism to systemic circ.
#         VMaxTCOH  VMax for hepatic TCOH->TCA (mg/hr)
#         KMTCOH              KM for hepatic TCOH->TCA (mg/L)
#         VMaxGluc  VMax for hepatic TCOH->TCOG (mg/hr)
#         KMGluc              KM for hepatic TCOH->TCOG (mg/L)
#         kMetTCOH  Rate constant for hepatic TCOH->other (/hr)
#         kUrnTCA             Rate constant for TCA plasma->urine (/hr)
#         kMetTCA             Rate constant for hepatic TCA->other (/hr)
#         kBile               Rate constant for TCOG liver->bile (/hr)
#         kEHR                Lumped rate constant for TCOG bile->TCOH liver (/hr)
#         kUrnTCOG  Rate constant for TCOG->urine (/hr)
#         kDCVG               Rate constant for hepatic DCVG->DCVC (/hr)
#         FracKidDCVC         Fraction of renal TCE GSH conj. "directly" to DCVC
#                             (i.e., via first pass)
#         VDCVG               DCVG effective volume of distribution
#         kNAT                Lumped rate constant for DCVC->Urinary NAcDCVC (/hr)
#         kKidBioact          Rate constant for DCVC bioactivation (/hr)
#         Rodents             Number of rodents in closed chamber data
#         VCh                 Chamber volume for closed chamber data
#         kLoss               Rate constant for closed chamber air loss
# Parameters used (not assigned here)
#         BW                  Body weight in kg
#         Species             1 = human (default), 2 = rat, 3 = mouse
#         Male                0 = female, 1 (default) = male
#         CC                  Closed chamber initial concentration
# Sampling/scaling parameters (assigned or sampled)
#         lnQCC
#         lnVPRC
#         lnDRespC
#         QFatC
#         QGutC
#         QLivC
#         QSlwC
#         QKidC
#         FracPlasC
#         VFatC
#         VGutC
#         VLivC
#         VRapC
#         VRespLumC
#         VRespEffC
#         VKidC
#         VBldC
#         lnPBC
#         lnPFatC
#         lnPGutC
#         lnPLivC
#         lnPRapC
#         lnPSlwC
#         lnPRespC
#         lnPKidC
#         lnPRBCPlasTCAC
#         lnPBodTCAC
#         lnPLivTCAC
#         lnkDissocC
#         lnBMaxkDC
#         lnPBodTCOHC
#         lnPLivTCOHC
#         lnPBodTCOGC
#         lnPLivTCOGC
#         lnPeffDCVG
#         lnkTSD
#         lnkAS
#         lnkTD
#         lnkAD
#         lnkASTCA
#         lnkASTCOH
#         lnVMaxC
#         lnKMC
#         lnClC
#         lnFracOtherC
#         lnFracTCAC
#         lnVMaxDCVGC
#         lnClDCVGC
#         lnKMDCVGC
#         lnVMaxKidDCVGC
#         lnClKidDCVGC
#         lnKMKidDCVGC
#         lnVMaxLungLivC
#         lnKMClara
#         lnFracLungSysC
#         lnVMaxTCOHC
#         lnClTCOHC
#         lnKMTCOH
#         lnVMaxGlucC
#         lnClGlucC
#         lnKMGluc
#         lnkMetTCOHC
#         lnkUrnTCAC
#         lnkMetTCAC
#         lnkBileC
#         lnkEHRC
#         lnkUrnTCOGC
#         lnFracKidDCVCC
#         lnkDCVGC
#         lnkNATC
#         lnkKidBioactC
#         NRodents
#         VChC
#         lnkLossC
# Input parameters
#         none
# Notes:
#*******************************************************************************
          # use measured value of > 0, otherwise use 0.03 for mouse,
          #         0.3 for rat, 60 for female human, 70 for male human
```

```
          BW = (BWmeas > 0.0 ? BWmeas : (Species == 3 ? 0.03 : (Species == 2 ? 0.3 :
(Male == 0 ? 60.0 : 70.0) )));

          BW75 = pow(BW, 0.75);
          BW25 = pow(BW, 0.25);

# Cardiac Output and alveolar ventilation (L/hr)
          QC = exp(lnQCC) * BW75 *      # Mouse, Rat, Human (default)
                    (Species == 3 ? 11.6 : (Species == 2 ? 13.3 : 16.0 ));
          # Mouse: CO=13.98 +/- 2.85 ml/min, BW=30 g (Brown et al. 1997, Tab. 22)
          #         Uncertainty CV is 0.20
          # Rat: CO=110.4 ml/min +/- 15.6, BW=396 g (Brown et al. 1997, Tab. 22,
          #         p 441).  Uncertainty CV is 0.14.
          # Human: Average of Male CO=6.5 l/min, BW=73 kg
          #         and female CO= 5.9 l/min, BW=60 kg (ICRP #89, sitting at rest)
          #         From Price et al. 2003, estimates of human perfusion rate were
          #         4.7~6.5 for females and 5.5~7.1 l/min for males (note
          #         portal blood was double-counted, and subtracted off here)
          #         Thus for uncertainty use CV of 0.2, truncated at 4xCV
          #         Variability from Price et al. (2003) had CV of 0.14~0.20,
          #         so use 0.2 as central estimate
          VPR = exp(lnVPRC)*
                    (Species == 3 ? 2.5 : (Species == 2 ? 1.9 : 0.96 ));
          # Mouse: QP/BW=116.5 ml/min/100 g (Brown et al. 1997, Tab. 31), VPR=2.5
          #         Assume uncertainty CV of 0.2 similar to QC, truncated at 4xCV
          #         Consistent with range of QP in Tab. 31
          # Rat: QP/BW=52.9 ml/min/100 g (Brown et al. 1997, Tab. 31), VPR=1.9
          #         Assume uncertainty CV of 0.3 similar to QC, truncated at 4xCV
          #         Used larger CV because Tab. 31 shows a very large range of QP
          # Human: Average of Male VE=9 l/min, resp. rate=12 /min,
          #         dead space=0.15 l (QP=7.2 l/min), and Female
          #         VE=6.5 l/min, resp. rate=14 /min, dead space=0.12 l
          #         (QP=4.8 l/min), VPR = 0.96
          #         Assume uncertainty CV of 0.2 similar to QC, truncated at 4xCV
          #         Consistent with range of QP in Tab. 31
          QPsamp = QC*VPR;

#         Respiratory diffusion flow rate
#         Will be scaled by QP in dynamics
#         Use log-uniform distribution from 1e-5 to 10
          DResptmp = exp(lnDRespC);

# Fractional Flows scaled to the appropriate species
# Fat = Adipose only
# Gut = GI tract + pancreas + spleen (all drain to portal vein)
# Liv = Liver, hepatic artery
# Slw = Muscle + Skin
# Kid = Kidney
# Rap = Rapidly perfused (rest of organs, plus bone marrow, lymph, etc.),
#         derived by difference in dynamics
#
# Mouse and rat data from Brown et al. (1997).  Human data from
#         ICRP-89 (2002), and is sex-specific.

          QFatCtmp = QFatC*
          (Species == 3 ? 0.07 : (Species == 2 ? 0.07 : (Male == 0 ? 0.085 : 0.05)
));
          QGutCtmp = QGutC*
          (Species == 3 ? 0.141 : (Species == 2 ? 0.153 : (Male == 0 ? 0.21 : 0.19)
));
          QLivCtmp = QLivC*
          (Species == 3 ? 0.02 : (Species == 2 ? 0.021 : 0.065 ));
          QSlwCtmp = QSlwC*
          (Species == 3 ? 0.217 : (Species == 2 ? 0.336 : (Male == 0 ? 0.17 : 0.22)
));
          QKidCtmp = QKidC*
                    (Species == 3 ? 0.091 : (Species == 2 ? 0.141 : (Male == 0 ?
0.17 : 0.19) ));

# Plasma Flows to Tissues (L/hr)
## Mice and rats from Hejtmancik et al. 2002,
##        control F344 rats and B6C3F1 mice at 19 weeks of age
## However, there appear to be significant strain differences in rodents, so
##        assume uncertainty CV=0.2 and variability CV=0.2.
## Human central estimate from ICRP.  Well measured in humans, from Price et al.,
##        human SD in hematocrit was 0.029 in females, 0.027 in males,
##        corresponding to FracPlas CV of 0.047 in females and
##        0.048 in males.  Use rounded CV = 0.05 for both uncertainty and
variability
## Use measured 1-hematocrit if available
## Truncate distributions at 3xCV to encompass clinical "normal range"
          FracPlas = (Hematocritmeas > 0.0 ? (1-Hematocritmeas) : (FracPlasC *
          (Species == 3 ? 0.52 : (Species == 2 ? 0.53 : (Male == 0 ? 0.615 :
0.567)))));

# Tissue Volumes (L)
# Fat = Adipose only
# Gut = GI tract (not contents) + pancreas + spleen (all drain to portal vein)
# Liv = Liver
# Rap = Brain + Heart + (Lungs-TB) + Bone marrow + "Rest of the body"
# VResp = Tracheobroncial region (trachea+broncial basal+
#                   broncial secretory+bronchiolar)
# Kid = Kidney
# Bld = Blood
# Slw = Muscle + Skin, derived by difference
# residual (assumed unperfused) = (Bone-Marrow)+GI contents+other
#
# Mouse and rat data from Brown et al. (1997).  Human data from
#         ICRP-89 (2002), and is sex-specific.

        VFat = BW * (VFatCmeas > 0.0 ? VFatCmeas : (VFatC * (Species == 3 ? 0.07 :
(Species == 2 ? 0.07 : (Male == 0 ? 0.317 : 0.199)  ))));
        VGut = VGutC * BW *
          (Species == 3 ? 0.049 : (Species == 2 ? 0.032 : (Male == 0 ? 0.022 :
0.020) ));
        VLiv = VLivC * BW *
```

```
          (Species == 3 ? 0.055 : (Species == 2 ? 0.034 : (Male == 0 ? 0.023 :
0.025) ));
        VRap = VRapC * BW *
          (Species == 3 ? 0.100 : (Species == 2 ? 0.088 : (Male == 0 ? 0.093 :
0.088) ));
          VRespLum = VRespLumC * BW *
          (Species == 3 ? (0.00014/0.03) : (Species == 2 ? (0.0014/0.3) : (0.167/70)
)); # Lumenal volumes from Styrene model (Sarangapani et al. 2002)
          VRespEfftmp = VRespEffC * BW *
          (Species == 3 ? 0.0007 : (Species == 2 ? 0.0005 : 0.00018 ));
          # Respiratory tract volume is TB region
          # will be multiplied by partition coef. below
          VKid = VKidC * BW *
          (Species == 3 ? 0.017 : (Species == 2 ? 0.007 : (Male == 0 ? 0.0046 :
0.0043) ));
        VBld = VBldC * BW *
          (Species == 3 ? 0.049 : (Species == 2 ? 0.074 : (Male == 0 ? 0.068 :
0.077) ));
        VSlw = (Species == 3 ? 0.8897 : (Species == 2 ? 0.8995 : (Male == 0 ?
0.85778 : 0.856))) * BW
                    - VFat - VGut - VLiv - VRap - VRespEfftmp - VKid - VBld;
# Slowly perfused:
# Baseline mouse: 0.8897-0.049-0.017-0.0007-0.1-0.055-0.049-0.07= 0.549
# Baseline rat: 0.8995 -0.074-0.007-0.0005-0.088-0.034-0.032-0.07= 0.594
# Baseline human F: 0.85778-0.068-0.0046-0.00018-0.093-0.023-0.022-0.317= 0.33
# Baseline human M: 0.856-0.077-0.0043-0.00018-0.088-0.025-0.02-0.199= 0.4425

       VPlas = FracPlas * VBld;
          VBod = VFat + VGut + VRap + VRespEfftmp + VKid + VSlw; # For TCA
          VBodTCOH = VBod + VBld;       # for TCOH and TCOG -- body without liver

# Partition coefficients
       PB = (PBmeas > 0.0 ? PBmeas : (exp(lnPBC) * (Species == 3 ? 15. : (Species ==
2 ? 22. : 9.5 )))); # Blood-air
          # Mice: pooling Abbas and Fisher 1997, Fisher et al. 1991
          #         each a single measurement, with overall CV = 0.07.
          #         Given small number of measurements, and variability
          #         in rat, use CV of 0.25 for uncertainty and variability.
          # Rats: pooling Sato et al. 1977, Gargas et al. 1989,
          #         Barton et al. 1995, Simmons et al. 2002, Koizumi 1989,
          #         Fisher et al. 1989.  Fisher et al. measurement substantially
          #         smaller than others (15 vs. 21~26).  Recent article
          #         by Rodriguez et al. 2007 shows significant change with
          #         age (13.1 at PND10, 17.5 at adult, 21.8 at aged), also seems
          #         to favor lower values than previously reported.  Therefore
          #         use CV = 0.25 for uncertainty and variability.
          # Humans: pooling Sato and Nakajima 1979, Sato et al. 1977,
          #         Gargas et al. 1989, Fiserova-Bergerova et al. 1984,
          #         Fisher et al. 1998, Koizumi 1989
          #         Overall variability CV = 0.185.  Consistent with
          #         within study inter-individual variability CV = 0.07~0.22.
          #         Study-to-study, sex-specific means range 8.1~11, so
          #         uncertainty CV = 0.2.
PFat = exp(lnPFatC) *            # Fat/blood
          (Species == 3 ? 36. : (Species == 2 ? 27. : 67. ));
   # Mice: Abbas and Fisher 1997.  Single measurement.  Use
   #         rat uncertainty of CV = 0.3.
   # Rats: Pooling Barton et al. 1995, Sato et al. 1977,
   #         Fisher et al. 1989.  Recent article by Rodriguez et al.
   #         (2007) shows higher value of 36., so assume uncertainty
   #         CV of 0.3.
   # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998,
   #         Sato et al. 1977.  Variability in Fat:Air has CV = 0.07.
   #         For uncertainty, dominated by PB uncertainty CV = 0.2
   #         For variability, add CVs in quadrature for
   #         sqrt(0.07^2+0.185^2)=0.20
PGut = exp(lnPGutC) *            # Gut/blood
          (Species == 3 ? 1.9 : (Species == 2 ? 1.4 : 2.6 ));
   # Mice: Geometric mean of liver, kidney
   # Rats: Geometric mean of liver, kidney
   # Humans: Geometric mean of liver, kidney
   #         Uncertainty of CV = 0.4 due to tissue extrapolation
PLiv = exp(lnPLivC) *            # Liver/blood
          (Species == 3 ? 1.7 : (Species == 2 ? 1.5 : 4.1 ));
   # Mice: Fisher et al. 1991, single datum, so assumed uncert CV = 0.4
   # Rats: Pooling Barton et al. 1995, Sato et al. 1977,
   #         Fisher et al. 1989, with little variation (range 1.3~1.7).
   #         Recent article by Rodriguez et al.reports 1.34.  Use
   #         uncertainty CV = 0.15.
   # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
   #         almost 2-fold difference in Liver:Air values, so uncertainty
   #         CV = 0.4
PRap = exp(lnPRapC) *            # Rapidly perfused/blood
          (Species == 3 ? 1.9 : (Species == 2 ? 1.3 : 2.6 ));
   # Mice: Similar to liver, kidney.  Uncertainty CV = 0.4 due to
   #         tissue extrapolation
   # Rats: Use brain values Sato et al. 1977.  Recent article by
   #         Rodriguez et al. (2007) reports 0.99 for brain.  Uncertainty
   #         CV of 0.4 due to tissue extrapolation.
   # Humans: Use brain from Fiserova-Bergerova et al. 1984
   #         Uncertainty of CV = 0.4 due to tissue extrapolation
PResp = exp(lnPRespC) *                    # Resp/blood =
          (Species == 3 ? 2.6 : (Species == 2 ? 1.0 : 1.3 ));
   # Mice: Abbas and Fisher 1997, single datum, so assumed uncert CV = 0.4
   # Rats: Sato et al. 1977, single datum, so assumed uncert CV = 0.4
   # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
   #         > 2-fold difference in lung:air values, so uncertainty
   #         CV = 0.4
VRespEff = VRespEfftmp * PResp * PB; # Effective air volume
PKid = exp(lnPKidC) *            # Slowly perfused/blood
          (Species == 3 ? 2.1 : (Species == 2 ? 1.3 : 1.6 ));
   # Mice: Abbas and Fisher 1997, single datum, so assumed uncert CV = 0.4
   # Rats: Pooling Barton et al. 1995, Sato et al. 1977.  Recent article
   #         by Rodriguez et al. (2007) reports 1.01, so use uncertainty
   #         CV of 0.3.  Pooled variability CV = 0.39.
   # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
```

```
        #        For uncertainty, dominated by PB uncertainty CV = 0.2
        #        Variability in kidney:air CV = 0.23, so add to PB variability
        #        in quadrature       sqrt(0.23^2+0.185^2)=0.30
     PSlw = exp(lnPSlwC) *           # Slowly perfused/blood
                 (Species == 3 ? 2.4 : (Species == 2 ? 0.58 : 2.1 ));
        # Mice: Muscle - Abbas and Fisher 1997, single datum, so assumed
        #        uncert CV = 0.4
        # Rats: Pooling Barton et al. 1995, Sato et al. 1977,
        #        Fisher et al. 1989.  Recent article by Rodriguez et al. (2007)
        #        reported 0.72, so use uncertainty CV of 0.25.  Variability
        #        in Muscle:air and muscle:blood ~ CV = 0.3
        # Humans: Pooling Fiserova-Bergerova et al. 1984, Fisher et al. 1998
        #        Range of values 1.4~2.4, so uncertainty CV = 0.3
        #        Variability in muscle:air CV = 0.3, so add to PB variability
        #        in quadrature sqrt(0.3^2+0.185^2)=0.35

# TCA partitioning
    TCAPlas = FracPlas + (1 - FracPlas) * 0.5 * exp(lnPRBCPlasTCAC);
        #        Blood/Plasma concentration ratio.  Note dependence
        #        on fraction of blood that is plasma.  Here
        #        exp(lnPRBCPlasTCA) = partition coefficient
        #                 C(blood minus plasma)/C(plasma)
        #        Default of 0.5, corresponding to Blood/Plasma
        #                 concentration ratio of 0.76 in
        #                 rats (Schultz et al 1999)
        #        For rats, Normal uncertainty with GSD = 1.4
        #        For mice and humans, diffuse prior uncertainty of
        #        100-fold up/down
    PBodTCA = TCAPlas * exp(lnPBodTCAC) *
                 (Species == 3 ? 0.88 : (Species == 2 ? 0.88 : 0.52 ));
        # Note -- these were done at 10~20 microg/ml (Abbas and Fisher 1997),
        #        which is 1.635-3.27 mmol/ml (1.635-3.27 x 10^6 microM).
        #        At this high concentration, plasma binding should be
        #        saturated -- e.g., plasma albumin concentration was
        #        measured to be P=190-239 microM in mouse, rat, and human
        #        plasma by Lumpkin et al. 2003, or > 6800 molecules of
        #        TCA per molecule of albumin.  So the measured partition
        #        coefficients should reflect free blood-tissue partitioning.
        # Used muscle values, multiplied by blood:plasma ratio to get
        #        Body:Plasma partition coefficient
        # Rats = mice from Abbas and Fisher 1997
        # Humans from Fisher et al. 1998
        #        Uncertainty in mice, humans GSD = 1.4
        #        For rats, GSD = 2.0, based on difference between mice
        #        and humans.
    PLivTCA = TCAPlas * exp(lnPLivTCAC) *
                 (Species == 3 ? 1.18 : (Species == 2 ? 1.18 : 0.66 ));
        # Multiplied by blood:plasma ratio to get Liver:Plasma
        # Rats = mice from Abbas and Fisher 1997
        # Humans from Fisher et al. 1998
        #        Uncertainty in mice, humans GSD = 1.4
        #        For rats, GSD = 2.0, based on difference between mice
        #        and humans.
```

```
# Binding Parameters for TCA
          # GM of Lumpkin et al. 2003; Schultz et al. 1999;
          #         Templin et al. 1993, 1995; Yu et al. 2000
          # Protein/TCA dissociation constant (umole/L)
          #         note - GSD = 3.29, 1.84, and 1.062 for mouse, rat, human
          kDissoc = exp(lnkDissocC) *
                    (Species == 3 ? 107. : (Species == 2 ? 275. : 182. ));
          # BMax = NSites * Protein concentration.  Sampled parameter is
          #         BMax/kD (determines binding at low concentrations)
          #         note - GSD = 1.64, 1.60, 1.20 for mouse, rat, human
          BMax = kDissoc * exp(lnBMaxkDC) *
                    (Species == 3 ? 0.88 : (Species == 2 ? 1.22 : 4.62 ));

# TCOH partitioning
          # Data from Abbas and Fisher 1997 (mouse) and Fisher et al.
          #         1998 (human).  For rat, used mouse values.
          #         Uncertainty in mice, humans GSD = 1.4
          #         For rats, GSD = 2.0, based on difference between mice
          #         and humans.

    PBodTCOH = exp(lnPBodTCOHC) *
                    (Species == 3 ? 1.11 : (Species == 2 ? 1.11 : 0.91 ));
    PLivTCOH = exp(lnPLivTCOHC) *
                    (Species == 3 ? 1.3 : (Species == 2 ? 1.3 : 0.59 ));

# TCOG partitioning
          # Use TCOH as a proxy, but uncertainty much greater
          # (e.g., use uniform prior, 100-fold up/down)
    PBodTCOG = exp(lnPBodTCOGC) *
                    (Species == 3 ? 1.11 : (Species == 2 ? 1.11 : 0.91 ));
    PLivTCOG = exp(lnPLivTCOGC) *
                    (Species == 3 ? 1.3 : (Species == 2 ? 1.3 : 0.59 ));

# DCVG distribution volume
          # exp(lnPeffDCVG) is the effective partition coefficient for
          # the "body" (non-blood) compartment
          # Diffuse prior distribution: loguniform 1e-3 to 1e3
          VDCVG = VBld +      # blood plus body (with "effective" PC)
          exp(lnPeffDCVG) * (VBod + VLiv);

# Absorption Rate Constants (/hr)
          # All priors are diffuse (log)uniform distributions
          # transfer from stomach centered on 1.4/hr, range up or down 100-fold,
          #         based on human stomach half-time of 0.5 hr.
        kTSD = exp(lnkTSD);
          # stomach absorption centered on 1.4/hr, range up or down 1000-fold
          kAS = exp(lnkAS);
          # assume no fecal excretion -- 100% absorption
          kTD = 0.0 * exp(lnkTD);
          # intestinal absorption centered on 0.75/hr, range up or down
          #         1000-fold, based on human transit time of small intestine
          #         of 4 hr   (95% throughput in 4 hr)
```

```
          kAD = exp(lnkAD);
          kASTCA = exp(lnkASTCA);
          kASTCOH = exp(lnkASTCOH);

# TCE Oxidative Metabolism Constants
# For rodents, in vitro microsomal data define priors (pooled).
# For human, combined in vitro microsomoal+hepatocellular individual data
#         define priors.
# All data from Elfarra et al. 1998; Lipscomb et al. 1997, 1998a,b
# For VMax, scaling from in vitro data were (Barter et al. 2007):
#         32 mg microsomal protein/g liver
#         99 x 1e6 hepatocytes/g liver
#         Here, human data assumed representative of mouse and rats.
# For KM, two different scaling methods were used for microsomes:
#         Assume microsomal concentration = liver concentration, and
#                   use central estimate of liver:blood PC (see above)
#         Use measured microsome:air partition coefficient (1.78) and
#                   central estimate of blood:air PC (see above)
# For human KM from hepatocytes, used measured human hepatocyte:air
#         partition coefficient (21.62, Lipscomb et al. 1998), and
#         central estimate of blood:air PC.
#         Note that to that the hepatocyte:air PC is similar to that
#         found in liver homogenates (human: 29.4+/-5.1 from Fiserova-
#         Bergerova et al. 1984, and 54 for Fisher et al. 1998; rat:
#         27.2+/-3.4 from Gargas et al. 1989, 62.7 from Koisumi 1989,
#         43.6 from Sato et al. 1977; mouse: 23.2 from Fisher et al. 1991).
# For humans, sampled parameters are VMax and ClC (VMax/KM), due to
#         improved convergence.  VMax is kept as a parameter because it
#         appears less uncertain (i.e., more consistent across microsomal
#         and hepatocyte data).

          # Central estimate of VMax is 342, 76.2, and 32.3 (micromol/min/
          #         kg liver) for mouse, rat, human.  Converting to /hr by
          #         * (60 min/hr * 0.1314 mg/micromol) gives
          #         2700, 600, and 255 mg/hr/kg liver
          # Observed variability of about 2-fold GSD.  Assume 2-fold GSD for
          #         both uncertainty and variability
        VMax = VLiv*exp(lnVMaxC)*
                    (Species == 3 ? 2700. : (Species == 2 ? 600. : 255.));

          # For mouse and rat central estimates for KM are 0.068~1.088 and
          #         0.039~0.679 mmol/l in blood, depending on the scaling
          #         method used.  Taking the geometric mean, and converting
          #         to mg/l by 131.4 mg/mmol gives 36. and 21. mg/l in blood.
          # For human, central estimate
          #         for Cl are 0.306~3.95 l/min/kg liver.  Taking the geometric
          #         mean and converting to /hr gives a central estimate of
          #         66. l/hr/kg.
          #         KM is then derived from KM = VMax/(Cl*Vliv) (central estimate
          #         of
          # Note uncertainty due to scaling is about 4-fold.
          #         Variability is about 3-fold in mice, 1.3-fold in rats, and
          #         2- to 4- fold in humans (depending on scaling).

        KM = (Species == 3 ? 36.*exp(lnKMC) : (Species == 2 ? 21.*exp(lnKMC) :
VMax/(VLiv*66.*exp(lnClC))));

# Oxidative metabolism splits
          # Fractional split of TCE to DCA
          # exp(lnFracOtherC) = ratio of DCA to non-DCA
          # Diffuse prior distribution: loguniform 1e-4 to 1e2
          FracOther = exp(lnFracOtherC)/(1+exp(lnFracOtherC));
          # Fractional split of TCE to TCA
          # exp(lnFracTCAC) = ratio of TCA to TCOH
          # TCA/TCOH = 0.1 from Lipscomb et al. 1998 using fresh hepatocytes,
          # but TCA/TCOH ~ 1 from Bronley-DeLancey et al 2006
          # GM = 0.32, GSD = 3.2
          FracTCA = 0.32*exp(lnFracTCAC)*(1-FracOther)/(1+0.32*exp(lnFracTCAC));

# TCE GSH Metabolism Constants
# Human in vitro data from Lash et al. 1999, define human priors.
#                             VMax (nmol/min/     KM (mM)             CLeff (ml/min/
#                                   g tissue)                               g tissue)
#                       ----------------------------------------------
#                             [high affinity pathway only]  [total]
# Human liver cytosol:        ~423                0.0055~0.023        21.2~87.0
# Human liver cytosol+        ~211                --                  --
#         microsomes
#                             [total]             [total]             [total]
# Human hepatocytes*          12~30**             0.012~0.039***      0.2~0.5****
# Human kidney cytosol:       81                  0.0164~0.0263       3.08~4.93
#         * estimated visually from Fig 1, Lash et al. 1999
#         ** Fig 1A, data from 50~500 ppm headspace at 60 min
#                   and Fig 1B, data at 100~5000 ppm in headspace for 120 min
#         *** Fig 1B, 30~100 ppm headspace, converted to blood concentration
#                   using blood:air PC of 9.5
#         **** Fig 1A, data at 50 ppm headspace at 120 min and Fig 1B, data at
#                   25 and 50 ppm headspace at 120 min.
# Overall, human liver hepatocytes are probably most like the
#         intact liver (e.g., accounting for the competition between
#         GSH conjugation and oxidation).  So central estimates based
#         on those: CLeff ~ 0.32 ml/min/g tissue, KM ~ 0.022 mM in blood.
#         CLeff converted to 19 l/hr/kg; KM converted to 2.9 mg/l in blood
#         However, uncertainty in CLeff is large (values in cytosol
#         ~100-fold larger).  Moreover, Green et al. 1997 reported
#         DCVG formation in cytosol that was ~30,000-fold smaller
#         than Lash et al. (1998) in cytosol, which would be a VMax
#         ~300-fold smaller than Lash et al. (1998) in hepatocytes.
#         Uncertainty in KM appears smaller (~4-fold)
#         CLC: GM = 19., GSD = 100; KM: GM = 2.9., GSD = 4.
#         In addition, at a single concentration, the variability
#         in human liver cytosol samples had a GSD=1.3.
# For the human kidney, the kidney cytosol values are used, with the same
#         uncertainty as for the liver.  Note that the DCVG formation rates
#         in rat kidney cortical cells and rat cytosol are quite similar
#         (see below).
#         CLC: GM = 230., GSD = 100; KM: GM = 2.7., GSD = 4.
```

```
# Rat and mouse in vitro data from Lash et al. 1995,1998 define rat and mouse
#         priors.  However, rats and mice are only assayed at 1 and 2 mM
#         providing only a bound on VMax and very little data on KM.
#                           Rate at 2 mM        Equivalent          CLeff
#                                               blood conc.         at 2 mM
#                           (nmol/min/          (mM)                (ml/min/
#                           g tissue)                     g tissue)
#                     ---------------------------------------------
# Rat     hepatocytes:      4.4~16              2.0                 0.0022~0.0079
#         liver cytosol:    8.0~12              1.7~2.0             0.0040~0.0072
#         kidney cells:     0.79~1.1  2.2                 0.00036~0.00049
#         kidney cytosol:   0.53~0.75 1.1~2.0             0.00027~0.00068
# Mouse   liver cytosol:    36~40               1.1~2.0             0.018~0.036
#         kidney cytosol:   6.2~9.3             0.91~2.0  0.0031~0.0102
#
# In most cases, rates were increased over the same sex/species at 1 mM,
#         indicating VMax has not yet been reached.  The values between cells
#         and cytosol are more much consistent that in the human data.
#         These data therefore put a lower bound on VMax and a lower bound
#         on CLC.  To account for in vitro-in vivo uncertainty, the lower
#         bound of the prior distribution is set 100-fold below the central
#         estimate of the measurements here.  In addition, Green et al.
#         (1997) found values 100-fold smaller than Lash et al. 1995, 1998.
#         Therefore diffuse prior distributions set to 1e-2~1e4.
# Rat liver: Bound on VMax of 4.4~16, with GM of 8.4.  Converting to
#         mg/hr/kg tissue (* 131.4 ng/nmol * 60 min/hr * 1e3 g/kg / 1e6 mg/ng)
#         gives a central estimate of 66. mg/hr/kg tissue.  Bound on CL of
#         0.0022~0.0079, with GM of 0.0042.  Converting to l/hr/kg tissue
#         (* 60 min/hr) gives 0.25 l/hr/kg tissue.
# Rat kidney: Bound on VMax of 0.53~1.1, with GM of 0.76.  Converting
#         to mg/hr/kg tissue gives a central estimate of 6.0 mg/hr/kg.
#         Bound on CL of 0.00027~0.00068, with GM of 0.00043.  Converting
#         to l/hr/kg tissue gives 0.026 l/hr/kg tissue.
# Mouse liver: Bound on VMax of 36~40, with GM of 38.  Converting
#         to mg/hr/kg tissue gives a central estimate of 300. mg/hr/kg.
#         Bound on CL of 0.018~0.036, with GM of 0.025.  Converting
#         to l/hr/kg tissue gives 1.53 l/hr/kg tissue.
# Mouse kidney: Bound on VMax of 6.2~9.3, with GM of 7.6.  Converting
#         to mg/hr/kg tissue gives a central estimate of 60. mg/hr/kg.
#         Bound on CL of 0.0031~0.0102, with GM of 0.0056.  Converting
#         to l/hr/kg tissue gives 0.34 l/hr/kg tissue.

          VMaxDCVG = VLiv*(Species == 3 ? (300.*exp(lnVMaxDCVGC)) : (Species == 2 ?
(66.*exp(lnVMaxDCVGC)) : (2.9*19.*exp(lnClDCVGC+lnKMDCVGC))));
        KMDCVG = (Species == 3 ? (VMaxDCVG/(VLiv*1.53*exp(lnClDCVGC))) : (Species ==
2 ? (VMaxDCVG/(VLiv*0.25*exp(lnClDCVGC))) : 2.9*exp(lnKMDCVGC)));
          VMaxKidDCVG = VKid*(Species == 3 ? (60.*exp(lnVMaxKidDCVGC)) : (Species ==
2 ? (6.0*exp(lnVMaxKidDCVGC)) : (2.7*230.*exp(lnClKidDCVGC+lnKMKidDCVGC))));
        KMKidDCVG = (Species == 3 ? (VMaxKidDCVG/(VKid*0.34*exp(lnClKidDCVGC))) :
(Species == 2 ? (VMaxKidDCVG/(VKid*0.026*exp(lnClKidDCVGC))) :
2.7*exp(lnKMKidDCVGC)));

# TCE Metabolism Constants for Chloral Kinetics in Lung (mg/hr)
#         Scaled to liver VMax using data from Green et al. (1997)
#         in microsomal preparations (nmol/min/mg protein) at ~1 mM.
#         For humans, used detection limit of 0.03
#         Additional scaling by lung/liver weight ratio
#         from Brown et al. Table 21 (mouse and rat) or
#         ICRP Pub 89 Table 2.8 (Human female and male)
#         Uncertainty ~ 3-fold truncated at 3 GSD
   VMaxClara = exp(lnVMaxLungLivC) * VMax *
          (Species == 3 ? (1.03/1.87*0.7/5.5):(Species == 2 ?
(0.08/0.82*0.5/3.4):(0.03/0.33*(Male == 0 ? (0.42/1.4) : (0.5/1.8)))));
   KMClara = exp(lnKMClara);
# Fraction of Respiratory Metabolism that goes to system circulation
# (translocated to the liver)
   FracLungSys = exp(lnFracLungSysC)/(1 + exp(lnFracLungSysC));

# TCOH Metabolism Constants (mg/hr)
          # No in vitro data.  So use diffuse priors of
          #         1e-4 to 1e4 mg/hr/kg^0.75 for VMax
          #                   (4e-5 to 4000 mg/hr for rat),
          #         1e-4 to 1e4 mg/l for KM,
          #         and 1e-5 to 1e3 l/hr/kg^0.75 for Cl
          #                   (2e-4 to 2.4e4 l/hr for human)
          VMaxTCOH = BW75*
                    (Species == 3 ? (exp(lnVMaxTCOHC)) : (Species == 2 ?
(exp(lnVMaxTCOHC)) : (exp(lnClTCOHC+lnKMTCOH))));
          KMTCOH = exp(lnKMTCOH);
          VMaxGluc = BW75*
                    (Species == 3 ? (exp(lnVMaxGlucC)) : (Species == 2 ?
(exp(lnVMaxGlucC)) : (exp(lnClGlucC+lnKMGluc))));
          KMGluc = exp(lnKMGluc);
          # No in vitro data.  So use diffuse priors of
          #         1e-5 to 1e3 kg^0.25/hr (3.5e-6/hr to 3.5e2/hr for human)
          kMetTCOH = exp(lnkMetTCOHC) / BW25;

# TCA kinetic parameters
          # Central estimate based on GFR clearance per unit body weight
          #         10.0, 8.7, 1.8 ml/min/kg for mouse, rat, human
          #         (= 0.6, 0.522, 0.108 l/hr/kg) from Lin 1995.
          #         = CL_GFR / BW (BW=0.02 for mouse, 0.265 for rat, 70 for human)
          #         kUrn = CL_GFR / VPlas
          #         Diffuse prior with uncertainty of up,down 100-fold
          kUrnTCA = exp(lnkUrnTCAC) * BW / VPlas *
                    (Species == 3 ? 0.6 : (Species == 2 ? 0.522 : 0.108));
          # No in vitro data.  So use diffuse priors of
          #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
          kMetTCA = exp(lnkMetTCAC) / BW25;

# TCOG kinetic parameters
          # No in vitro data.  So use diffuse priors of
          #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
          kBile = exp(lnkBileC) / BW25;
        kEHR = exp(lnkEHRC) / BW25;
          # Central estimate based on GFR clearance per unit body weight
```

```
          #         10.0, 8.7, 1.8 ml/min/kg for mouse, rat, human
          #         (= 0.6, 0.522, 0.108 l/hr/kg) from Lin 1995.
          #         = CL_GFR / BW (BW=0.02 for mouse, 0.265 for rat, 70 for human)
          #         kUrn = CL_GFR / VBld
          #         Diffuse prior with Uncertainty of up,down 1000-fold
          kUrnTCOG = exp(lnkUrnTCOGC) * BW / (VBodTCOH * PBodTCOG) *
                    (Species == 3 ? 0.6 : (Species == 2 ? 0.522 : 0.108));

# DCVG Kinetics (/hr)
          # Fraction of renal TCE GSH conj. "directly" to DCVC via "first pass"
          # exp(lnFracOtherCC) = ratio of direct/non-direct
          # Diffuse prior distribution: loguniform 1e-3 to 1e3
          # FIXED in v1.2.3
          # In ".in" files, set to 1, so that all kidney GSH conjugation
          # is assumed to directly produce DCVC (model lacks identifiability
          # otherwise).
          FracKidDCVC = exp(lnFracKidDCVCC)/(1 + exp(lnFracKidDCVCC));
          # No in vitro data.  So use diffuse priors of
          #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
          kDCVG = exp(lnkDCVGC) / BW25;

# DCVC Kinetics in Kidney (/hr)
          # No in vitro data.  So use diffuse priors of
          #         1e-4 to 1e2 /hr/kg^0.25 (0.3/hr to 35/hr for human)
          kNAT = exp(lnkNATC) / BW25;
          kKidBioact = exp(lnkKidBioactC) / BW25;

# CC data initialization
          Rodents = (CC > 0 ? NRodents : 0.0); # Closed chamber simulation
          VCh = (CC > 0 ? VChC - (Rodents * BW) : 1.0);
                    # Calculate net chamber volume
          kLoss = (CC > 0 ? exp(lnkLossC) : 0.0);

#*******************************************************************************
#***                 State Variable Initialization and Scaling             ***
#*******************************************************************************
# NOTE: All State Variables are automatically set to 0 initially,
# unless re-initialized here

          ACh = (CC * VCh * MWTCE) / 24450.0;    # Initial amount in chamber

};
##################### End of Initialization #######################

Dynamics{

#*******************************************************************************
#***                      Dynamic physiological parameter scaling          ***
#*******************************************************************************
# State Variables with dynamics:
#         none
# Input Variables:
#         QPmeas
# Other State Variables and Global Parameters:
#         QC
#         VPR
#         DResptmp
#         QPsamp
#         QFatCtmp
#         QGutCtmp
#         QLivCtmp
#         QSlwCtmp
#         QKidCtmp
#         FracPlas
# Temporary variables used:
#         none
# Temporary variables assigned:
#         QP
#         DResp
#         QCnow
#         QFat
#         QGut
#         QLiv
#         QSlw
#         QKid
#         QGutLiv
#         QRap
#         QCPlas
#         QBodPlas
#         QGutLivPlas
# Notes:
#*******************************************************************************

# QP uses QPmeas if value is > 0, otherwise uses sampled value
          QP = (QPmeas > 0 ? QPmeas : QPsamp);
          DResp = DResptmp * QP;

# QCnow uses QPmeas/VPR if QPmeas > 0, otherwise uses sampled value
          QCnow = (QPmeas > 0 ? QPmeas/VPR : QC);

# These done here in dynamics in case QCnow changes
# Blood Flows to Tissues (L/hr)
        QFat = (QFatCtmp) * QCnow; #
        QGut = (QGutCtmp) * QCnow; #
        QLiv = (QLivCtmp) * QCnow; #
        QSlw = (QSlwCtmp) * QCnow; #

        QKid = (QKidCtmp) * QCnow; #
     QGutLiv = QGut + QLiv; #
          QRap = QCnow - QFat - QGut - QLiv - QSlw - QKid;
          QRapCtmp = QRap/QCnow; #(vrisk)
          QBod = QCnow - QGutLiv;

# Plasma Flows to Tissues (L/hr)
      QCPlas = FracPlas * QCnow; #
    QBodPlas = FracPlas * QBod; #
```

```
    QGutLivPlas = FracPlas * QGutLiv; #

#******************************************************************************
#***                  Exposure and Absorption calculations                  ***
#******************************************************************************
# State Variables with dynamics:
#         AStom
#         ADuod
#         AStomTCA
#         AStomTCOH
# Input Variables:
#         IVDose
#         PDose
#         Drink
#         Conc
#         IVDoseTCA
#         PODoseTCA
#         IVDoseTCOH
#         PODoseTCOH
# Other State Variables and Global Parameters:
#         ACh
#         CC
#         VCh
#         MWTCE
#         BW
#         TChng
#         kAS
#         kTSD
#         kAD
#         kTD
#         kASTCA
#         kASTCOH
# Temporary variables used:
#         none
# Temporary variables assigned:
#         kIV - rate into CVen
#         kIA - rate into CArt
#         kPV - rate into portal vein
#         kStom - rate into stomach
#         kDrink - incorporated into RAO
#         RAO - rate into gut (oral absorption - both gavage and drinking water)
#         CInh - inhalation exposure concentration
#         kIVTCA - rate into blood
#         kStomTCA - rate into stomach
#         kPOTCA - rate into liver (oral absorption)
#         kIVTCOH - rate into blood
#         kStomTCOH - rate into stomach
#         kPOTCOH - rate into liver (oral absorption)
# Notes:
# For oral dosing, using "Spikes" for instantaneous inputs
# Inhalation Concentration (mg/L)
#         CInh uses Conc when open chamber (CC=0) and
#         ACh/VCh when closed chamber CC>0.
```

```
#******************************************************************************

#### TCE DOSING
## IV route
    kIV = (IVDose * BW) / TChng;# IV infusion rate (mg/hr)
                                        # (IVDose constant for duration TChng)
    kIA = (IADose * BW) / TChng;        # IA infusion rate (mg/hr)
    kPV = (PVDose * BW) / TChng;        # PV infusion rate (mg/hr)
    kStom = (PDose * BW) / TChng;# PO dose rate (into stomach) (mg/hr)

## Oral route
# Amount of TCE in stomach -- for oral dosing only (mg)
    dt(AStom) = kStom - AStom * (kAS + kTSD);

# Amount of TCE in duodenum -- for oral dosing only (mg)
    dt(ADuod) = (kTSD * AStom) - (kAD + kTD) * ADuod;
# Rate of absorption from drinking water
    kDrink = (Drink * BW) / 24.0; #Ingestion rate via drinking water (mg/hr)
# Total rate of absorption including gavage and drinking water
    RAO = kDrink + (kAS * AStom) + (kAD * ADuod);
## Inhalation route
    CInh = (CC > 0 ? ACh/VCh : Conc*MWTCE/24450.0); # in mg/l

#### TCA Dosing
          kIVTCA = (IVDoseTCA * BW) / TChng;  # TCA IV infusion rate (mg/hr)
          kStomTCA = (PODoseTCA * BW) / TChng; # TCA PO dose rate into stomach
          dt(AStomTCA) = kStomTCA - AStomTCA * kASTCA;
          kPOTCA = AStomTCA * kASTCA;  # TCA oral absorption rate (mg/hr)

#### TCOH Dosing
          kIVTCOH = (IVDoseTCOH * BW) / TChng;#TCOH IV infusion rate (mg/hr)
          kStomTCOH = (PODoseTCOH * BW) / TChng; # TCOH PO dose rate into stomach
    dt(AStomTCOH) = kStomTCOH - AStomTCOH * kASTCOH;
          kPOTCOH = AStomTCOH * kASTCOH;# TCOH oral absorption rate (mg/hr)

#******************************************************************************
#***                          TCE Model                                     ***
#******************************************************************************
# State Variables with dynamics:
#         ARap,               # Amount in rapidly perfused tissues
#         ASlw,               # Amount in slowly perfused tissues
#         AFat,               # Amount in fat
#         AGut,               # Amount in gut
#         ALiv,               # Amount in liver
#         AInhResp,
#         AResp,
#         AExhResp,
#         AKid,               # Amount in Kidney -- currently in Rap tissue
#         ABld,               # Amount in Blood -- currently in Rap tissue
#         ACh,                # Amount of TCE in closed chamber
# Input Variables:
#         none
# Other State Variables and Global Parameters:
```

```
#         VRap
#         PRap
#         VSlw
#         PSlw
#         VFat
#         PFat
#         VGut
#         PGut
#         VLiv
#         PLiv
#         VRespLum
#         VRespEff
#         FracLungSys
#         VKid
#         PKid
#         VBld
#         VMaxClara
#         KMClara
#         PB
#         Rodents
#         VCh
#         kLoss
#         VMax
#         KM
#         VMaxDCVG
#         KMDCVG
#         VMaxKidDCVG
#         KMKidDCVG
# Temporary variables used:
#         QM
#         QFat
#         QGutLiv
#         QSlw
#         QRap
#         QKid
#         kIV
#         QCnow
#         CInh
#         QP
#         RAO
# Temporary variables assigned:
#         QM
#         CRap
#         CSlw
#         CFat
#         CGut
#         CLiv
#         CInhResp
#         CResp
#         CExhResp
#         ExhFactor
#         CMixExh
#         CKid
#         CVRap
#         CVSlw
#         CVFat
#         CVGut
#         CVLiv
#         CVTB
#         CVKid
#         CVen
#         RAMetLng
#         CArt_tmp
#         CArt
#         CAlv
#         RAMetLiv1
#         RAMetLiv2
#         RAMetKid
# Notes:
#******************************************************************************
#

#****Blood (venous)***********************************************************
# Tissue Concentrations (mg/L)
          CRap = ARap/VRap;
          CSlw = ASlw/VSlw;
          CFat = AFat/VFat;
          CGut = AGut/VGut;
          CLiv = ALiv/VLiv;
          CKid = AKid/VKid;
# Venous Concentrations (mg/L)
          CVRap = CRap / PRap;
          CVSlw = CSlw / PSlw;
          CVFat = CFat / PFat;
          CVGut = CGut / PGut;
          CVLiv = CLiv / PLiv;
          CVKid = CKid / PKid;
# Concentration of TCE in mixed venous blood (mg/L)
          CVen = ABld/VBld;
# Dynamics for blood
    dt(ABld) = (QFat*CVFat + QGutLiv*CVLiv + QSlw*CVSlw +
                    QRap*CVRap + QKid*CVKid + kIV) - CVen * QCnow;

#****Gas exchange and Respiratory Metabolism**********************************
#
    QM = QP/0.7;    # Minute-volume
    CInhResp = AInhResp/VRespLum;
    CResp = AResp/VRespEff;
    CExhResp = AExhResp/VRespLum;
    dt(AInhResp) = (QM*CInh + DResp*(CResp-CInhResp) - QM*CInhResp);
    RAMetLng = VMaxClara * CResp/(KMClara + CResp);
    dt(AResp) = (DResp*(CInhResp + CExhResp - 2*CResp) - RAMetLng);
    CArt_tmp = (QCnow*CVen + QP*CInhResp)/(QCnow + (QP/PB));
    dt(AExhResp) = (QM*(CInhResp-CExhResp) + QP*(CArt_tmp/PB-CInhResp) +
                    DResp*(CResp-CExhResp));
    CMixExh = (CExhResp > 0 ? CExhResp : 1e-15); # mixed exhaled breath
```

```
# Concentration in alveolar air (mg/L)
          # Correction factor for exhaled air to account for
          # absorption/desorption/metabolism in respiratory tissue
          # = 1 if DResp = 0
          ExhFactor_den = (QP * CArt_tmp / PB + (QM-QP)*CInhResp);
          ExhFactor = (ExhFactor_den > 0) ? (
                    QM * CMixExh / ExhFactor_den) : 1;
          # End-exhaled breath (corrected for absorption/
          #         desorption/metabolism in respiratory tissue)
          CAlv = CArt_tmp / PB * ExhFactor;
# Concentration in arterial blood entering circulation (mg/L)
          CArt = CArt_tmp + kIA/QCnow;  # add inter-arterial dose

#****Other dynamics for inhalation/exhalation *********************************
# Dynamics for amount of TCE in closed chamber
    dt(ACh) = (Rodents * (QM * CMixExh - QM * ACh/VCh)) - (kLoss * ACh);

#**** Non-metabolizing tissues ************************************************
# Amount of TCE in rapidly perfused tissues (mg)
    dt(ARap) = QRap * (CArt - CVRap);
# Amount of TCE in slowly perfused tissues
    dt(ASlw) = QSlw * (CArt - CVSlw);
# Amount of TCE in fat tissue (mg)
    dt(AFat) = QFat*(CArt - CVFat);
# Amount of TCE in gut compartment (mg)
    dt(AGut) = (QGut * (CArt - CVGut)) + RAO;

#**** Liver *******************************************************************
# Rate of TCE oxidation by P450 to TCA, TCOH, and other (DCA) in liver (mg/hr)
          RAMetLiv1 = (VMax * CVLiv) / (KM + CVLiv);
# Rate of TCE metabolized to DCVG in liver (mg)
          RAMetLiv2 = (VMaxDCVG * CVLiv) / (KMDCVG + CVLiv);
# Dynamics for amount of TCE in liver (mg)
    dt(ALiv) = (QLiv * (CArt - CVLiv)) + (QGut * (CVGut - CVLiv))
                              - RAMetLiv1 - RAMetLiv2 + kPV; # added PV dose

#**** Kidney ******************************************************************
# Rate of TCE metabolized to DCVG in kidney (mg) #
          RAMetKid = (VMaxKidDCVG * CVKid) / (KMKidDCVG + CVKid);
# Amount of TCE in kidney compartment (mg)
    dt(AKid) = (QKid * (CArt - CVKid)) - RAMetKid;

#******************************************************************************
#***                       TCOH Sub-model                                   ***
#******************************************************************************
# State Variables with dynamics:
#         ABodTCOH
#         ALivTCOH
# Input Variables:
#         none
# Other State Variables and Global Parameters:
#         ABileTCOG
#         kEHR
#         VBodTCOH
#         PBodTCOH
#         VLiv
#         PLivTCOH
#         VMaxTCOH
#         KMTCOH
#         VMaxGluc
#         KMGluc
#         kMetTCOH - hepatic metabolism of TCOH (e.g., to DCA)
#         FracOther
#         FracTCA
#         StochTCOHTCE
#         StochTCOHGluc
#         FracLungSys
# Temporary variables used:
#         QBod
#         QGutLiv
#         QCnow
#         kPOTCOH
#         RAMetLiv1
#         RAMetLng
# Temporary variables assigned:
#         CVBodTCOH
#         CVLivTCOH
#         CTCOH
#         RAMetTCOHTCA
#         RAMetTCOHGluc
#         RAMetTCOH
#         RARecircTCOG
# Notes:
#******************************************************************************
#**** Blood (venous=arterial) *************************************************
# Venous Concentrations (mg/L)
          CVBodTCOH = ABodTCOH / VBodTCOH / PBodTCOH;
          CVLivTCOH = ALivTCOH / VLiv / PLivTCOH;
          CTCOH = (QBod * CVBodTCOH + QGutLiv * CVLivTCOH + kIVTCOH)/QCnow;

#**** Body ********************************************************************
# Amount of TCOH in body
    dt(ABodTCOH) = QBod * (CTCOH - CVBodTCOH);

#**** Liver *******************************************************************

# Rate of oxidation of TCOH to TCA (mg/hr)
          RAMetTCOHTCA = (VMaxTCOH * CVLivTCOH) / (KMTCOH + CVLivTCOH);
# Amount of glucuronidation to TCOG (mg/hr)
          RAMetTCOHGluc = (VMaxGluc * CVLivTCOH) / (KMGluc + CVLivTCOH);
# Amount of TCOH metabolized to other (e.g., DCA)
          RAMetTCOH = kMetTCOH * ALivTCOH;
# Amount of TCOH-Gluc recirculated (mg)
          RARecircTCOG = kEHR * ABileTCOG;
# Amount of TCOH in liver (mg)
```

```
    dt(ALivTCOH) = kPOTCOH + QGutLiv * (CTCOH - CVLivTCOH)
                    - RAMetTCOH - RAMetTCOHTCA - RAMetTCOHGluc
                    + ((1.0 - FracOther - FracTCA) * StochTCOHTCE *
                    (RAMetLiv1 + FracLungSys*RAMetLng))
                    + (StochTCOHGluc * RARecircTCOG);

#******************************************************************************
#***                        TCA Sub-model                                  ***
#******************************************************************************
# State Variables with dynamics:
#         APlasTCA
#         ABodTCA
#         ALivTCA
#         AUrnTCA
#         AUrnTCA_sat
#         AUrnTCA_collect
# Input Variables:
#         TCAUrnSat
#         UrnMissing
# Other State Variables and Global Parameters:
#         VPlas
#         MWTCA
#         kDissoc
#         BMax
#         kMetTCA -- hepatic metabolism of TCA (e.g., to DCA)
#         VBod
#         PBodTCA
#         PLivTCA
#         kUrnTCA
#         FracTCA
#         StochTCATCE
#         StochTCATCOH
#         FracLungSys
# Temporary variables used:
#         kIVTCA
#         kPOTCA
#         QBodPlas
#         QGutLivPlas
#         QCPlas
#         RAMetLiv1
#         RAMetTCOHTCA
#         RAMetLng
# Temporary variables assigned:
#         CPlasTCA
#         CPLasTCAMole
#         a, b, c
#         CPlasTCAFreeMole
#         CPlasTCAFree
#         APlasTCAFree
#         CPlasTCABnd
#         CBodTCAFree
#         CLivTCAFree
#         CBodTCA
#         CLivTCA
#         CVBodTCA
#         CVLivTCA
#         RUrnTCA
#         RAMetTCA
# Notes:
#******************************************************************************
#**** Plasma ******************************************************************
# Concentration of TCA in plasma (umoles/L)
          CPlasTCA = (APlasTCA<1.0e-15 ? 1.0e-15 : APlasTCA/VPlas);
# Concentration of free TCA in plasma in (umoles/L)
          CPlasTCAMole = (CPlasTCA / MWTCA) * 1000.0;
          a = kDissoc+BMax-CPlasTCAMole;
          b = 4.0*kDissoc*CPlasTCAMole;
          c = (b < 0.01*a*a ? b/2.0/a : sqrt(a*a+b)-a);
          CPlasTCAFreeMole = 0.5*c;
# Concentration of free TCA in plasma (mg/L)
          CPlasTCAFree = (CPlasTCAFreeMole * MWTCA) / 1000.0;
          APlasTCAFree = CPlasTCAFree * VPlas;
# Concentration of bound TCA in plasma (mg/L)
          CPlasTCABnd = (CPlasTCA<CPlasTCAFree ? 0 : CPlasTCA-CPlasTCAFree);
# Concentration in body and liver
          CBodTCA = (ABodTCA<0 ? 0 : ABodTCA/VBod);
          CLivTCA = (ALivTCA<1.0e-15 ? 1.0e-15 : ALivTCA/VLiv);
# Total concentration in venous plasma (free+bound)
          CVBodTCAFree = (CBodTCA / PBodTCA);     # free in equilibrium
          CVBodTCA = CPlasTCABnd + CVBodTCAFree;
          CVLivTCAFree = (CLivTCA / PLivTCA);
          CVLivTCA = CPlasTCABnd + CVLivTCAFree;  # free in equilibrium
# Rate of urinary excretion of TCA
          RUrnTCA = kUrnTCA * APlasTCAFree;
# Dynamics for amount of total (free+bound) TCA in plasma (mg)
    dt(APlasTCA) = kIVTCA + (QBodPlas*CVBodTCA) + (QGutLivPlas*CVLivTCA)
                              - (QCPlas * CPlasTCA) - RUrnTCA;

#**** Body ********************************************************************
# Dynamics for amount of TCA in the body (mg)
    dt(ABodTCA) = QBodPlas * (CPlasTCAFree - CVBodTCAFree);

#**** Liver *******************************************************************
# Rate of metabolism of TCA
          RAMetTCA = kMetTCA * ALivTCA;
# Dynamics for amount of TCA in the liver (mg)
    dt(ALivTCA) = kPOTCA + QGutLivPlas*(CPlasTCAFree - CVLivTCAFree)
                              - RAMetTCA + (FracTCA * StochTCATCE *
                              (RAMetLiv1 + FracLungSys*RAMetLng))
                              + (StochTCATCOH * RAMetTCOHTCA);

#**** Urine *******************************************************************
# Dynamics for amount of TCA in urine (mg)
    dt(AUrnTCA) = RUrnTCA;
    dt(AUrnTCA_sat) = TCAUrnSat*(1-UrnMissing)* RUrnTCA;
                    # Saturated, but not missing collection times
```

```
    dt(AUrnTCA_collect) = (1-TCAUrnSat)*(1-UrnMissing)*RUrnTCA;
                    # Not saturated and not missing collection times

#******************************************************************************
#***                       TCOG Sub-model                                  ***
#******************************************************************************
# State Variables with dynamics:
#         ABodTCOG
#         ALivTCOG
#         ABileTCOG
#         AUrnTCOG
#         AUrnTCOG_sat
#         AUrnTCOG_collect
# Input Variables:
#         TCOGUrnSat
#         UrnMissing
# Other State Variables and Global Parameters:
#         VBodTCOH
#         VLiv
#         PBodTCOG
#         PLivTCOG
#         kUrnTCOG
#         kBile
#         StochGlucTCOH
# Temporary variables used:
#         QBod
#         QGutLiv
#         QCnow
#         RAMetTCOHGluc
#         RARecircTCOG
# Temporary variables assigned:
#         CVBodTCOG
#         CVLivTCOG
#         CTCOG
#         RUrnTCOG
#         RBileTCOG
# Notes:
#******************************************************************************
#**** Blood (venous=arterial) *************************************************
# Venous Concentrations (mg/L)
          CVBodTCOG = ABodTCOG / VBodTCOH / PBodTCOG;
          CVLivTCOG = ALivTCOG / VLiv / PLivTCOG;
          CTCOG = (QBod * CVBodTCOG + QGutLiv * CVLivTCOG)/QCnow;
#**** Body ********************************************************************
# Amount of TCOG in body
          RUrnTCOG = kUrnTCOG * ABodTCOG;
    dt(ABodTCOG) = QBod * (CTCOG - CVBodTCOG) - RUrnTCOG;
          RUrnTCOGTCOH = RUrnTCOG*StochTCOHGluc; #(vrisk)
#**** Liver *******************************************************************
# Amount of TCOG in liver (mg)
          RBileTCOG = kBile * ALivTCOG;
    dt(ALivTCOG) = QGutLiv * (CTCOG - CVLivTCOG)
                    + (StochGlucTCOH * RAMetTCOHGluc) - RBileTCOG;
```

```
#**** Bile ********************************************************************
# Amount of TCOH-Gluc excreted into bile (mg)
    dt(ABileTCOG) = RBileTCOG - RARecircTCOG;

#**** Urine *******************************************************************
# Amount of TCOH-Gluc excreted in urine (mg)
    dt(AUrnTCOG) = RUrnTCOG;
    dt(AUrnTCOG_sat) = TCOGUrnSat*(1-UrnMissing)*RUrnTCOG;
                    # Saturated, but not missing collection times
    dt(AUrnTCOG_collect) = (1-TCOGUrnSat)*(1-UrnMissing)*RUrnTCOG;
                    # Not saturated and not missing collection times

#******************************************************************************
#***                       DCVG Sub-model                                  ***
#******************************************************************************
# State Variables with dynamics:
#         ADCVGmol
# Input Variables:
#         none
# Other State Variables and Global Parameters:
#         kDCVG
#         FracKidDCVC         # Fraction of kidney DCVG going to DCVC in first pass
#         VDCVG
# Temporary variables used:
#         RAMetLiv2
#         RAMetKid
# Temporary variables assigned:
#         RAMetDCVGmol
#         CDCVGmol
# Notes:
#         Assume negligible GGT activity in liver as compared to kidney,
#         supported by in vitro data on GGT (even accounting for 5x
#         greater liver mass relative to kidney mass), as well as lack
#         of DCVC detected in blood.
#         "FracKidDCVC" Needed to account for "first pass" in
#         kidney (TCE->DCVG->DCVC without systemic circulation of DCVG).
#******************************************************************************
# Rate of metabolism of DCVG to DCVC
          RAMetDCVGmol = kDCVG * ADCVGmol;
# Dynamics for DCVG in blood
    dt(ADCVGmol) = (RAMetLiv2 + RAMetKid*(1-FracKidDCVC)) / MWTCE
                    - RAMetDCVGmol;
# Concentration of DCVG in blood (in mmoles/l)
          CDCVGmol = ADCVGmol / VDCVG;

#******************************************************************************
#***                       DCVC Sub-model                                  ***
#******************************************************************************
# State Variables with dynamics:
#         ADCVC
#         AUrnNDCVC
# Input Variables:
```

```
#         none
# Other State Variables and Global Parameters:
#         MWDCVC
#         FracKidDCVC
#         StochDCVCTCE
#         kNAT
#         kKidBioact
#         StochN
# Temporary variables used:
#         RAMetDCVGmol
#         RAMetKid
# Temporary variables assigned:
#         RAUrnDCVC
# Notes:
#         Cannot detect DCVC in blood, so assume all is locally generated
#         and excreted or bioactivated in kidney.
#******************************************************************************
# Amount of DCVC in kidney (mg)
    dt(ADCVC) = RAMetDCVGmol * MWDCVC
                    + RAMetKid * FracKidDCVC * StochDCVCTCE
                    - ((kNAT + kKidBioact) * ADCVC);
# Rate of NAcDCVC excretion into urine (mg)
          RAUrnDCVC = kNAT * ADCVC;
# Dynamics for amount of N Acetyl DCVC excreted (mg)
     dt(AUrnNDCVC) = StochN * RAUrnDCVC;
          RUrnNDCVC = StochN * RAUrnDCVC; #(vrisk)
#******************************************************************************
#***                       Total Mass Balance                                  ***
#******************************************************************************
#**** Mass Balance for TCE ****************************************************
# Total intake from inhalation (mg)
          RInhDose = QM * CInh;
    dt(InhDose) = RInhDose;
# Amount of TCE absorbed by non-inhalation routes (mg)
    dt(AO) = RAO + kIV + kIA + kPV; #(vrisk)
# Total dose
          TotDose = InhDose + AO; #(vrisk)
# Total in tissues
          TotTissue = #(vrisk)
                    ARap + ASlw + AFat + AGut + ALiv + AKid + ABld + #(vrisk)
                    AInhResp + AResp + AExhResp; #(vrisk)
# Total metabolized
    dt(AMetLng) = RAMetLng; #(vrisk)
    dt(AMetLiv1) = RAMetLiv1; #(vrisk)
    dt(AMetLiv2) = RAMetLiv2; #(vrisk)
    dt(AMetKid) = RAMetKid; #(vrisk)
          ATotMetLiv = AMetLiv1 + AMetLiv2; #(vrisk)
          TotMetab = AMetLng + ATotMetLiv + AMetKid; #(vrisk)
          AMetLivOther = AMetLiv1 * FracOther; #(vrisk)
          AMetGSH = AMetLiv2 + AMetKid; #(vrisk)
# Amount of TCE excreted in feces (mg)
          RAExc = kTD * ADuod; #(vrisk)
    dt(AExc) = RAExc; #(vrisk)
# Amount exhaled (mg)
          RAExh = QM * CMixExh;
    dt(AExh) = RAExh;
# Mass balance
          TCEDiff = TotDose - TotTissue - TotMetab; #(vrisk)
          MassBalTCE = TCEDiff - AExc - AExh; #(vrisk)

#**** Mass Balance for TCOH ****************************************************
# Total production/intake of TCOH
    dt(ARecircTCOG) = RARecircTCOG; #(vrisk)
    dt(AOTCOH) = kPOTCOH + kIVTCOH; #(vrisk)
          TotTCOHIn = AOTCOH + ((1.0 - FracOther - FracTCA) * #(vrisk)
                    StochTCOHTCE * (AMetLiv1 + FracLungSys*AMetLng)) + #(vrisk)
                    (StochTCOHGluc * ARecircTCOG); #(vrisk)
          TotTCOHDose = AOTCOH + ((1.0 - FracOther - FracTCA) * #(vrisk)
                    StochTCOHTCE * (AMetLiv1 + FracLungSys*AMetLng)); #(vrisk)
# Total in tissues
          TotTissueTCOH = ABodTCOH + ALivTCOH; #(vrisk)
# Total metabolism of TCOH
    dt(AMetTCOHTCA) = RAMetTCOHTCA; #(vrisk)
    dt(AMetTCOHGluc) = RAMetTCOHGluc; #(vrisk)
    dt(AMetTCOHOther) = RAMetTCOH; #(vrisk)
          TotMetabTCOH = AMetTCOHTCA + AMetTCOHGluc + AMetTCOHOther; #(vrisk)
# Mass balance
          MassBalTCOH = TotTCOHIn - TotTissueTCOH - TotMetabTCOH; #(vrisk)

#**** Mass Balance for TCA ****************************************************
# Total production/intake of TCA
    dt(AOTCA) = kPOTCA + kIVTCA; #(vrisk)
          TotTCAIn = AOTCA + (FracTCA*StochTCATCE*(AMetLiv1 + #(vrisk)
                    FracLungSys*AMetLng)) + (StochTCATCOH*AMetTCOHTCA); #(vrisk)
# Total in tissues
          TotTissueTCA = APlasTCA + ABodTCA + ALivTCA; #(vrisk)
# Total metabolism of TCA
    dt(AMetTCA) = RAMetTCA; #(vrisk)
# Mass balance
          TCADiff = TotTCAIn - TotTissueTCA - AMetTCA; #(vrisk)
          MassBalTCA = TCADiff - AUrnTCA; #(vrisk)

#**** Mass Balance for TCOG ****************************************************
# Total production of TCOG
          TotTCOGIn = StochGlucTCOH * AMetTCOHGluc; #(vrisk)
# Total in tissues
          TotTissueTCOG = ABodTCOG + ALivTCOG + ABileTCOG; #(vrisk)
# Mass balance
          MassBalTCOG = TotTCOGIn - TotTissueTCOG - #(vrisk)
                    ARecircTCOG - AUrnTCOG; #(vrisk)

#**** Mass Balance for DCVG ****************************************************
# Total production of DCVG
    dt(ADCVGIn) = (RAMetLiv2 + RAMetKid*(1-FracKidDCVC)) / MWTCE; #(vrisk)
# Metabolism of DCVG
    dt(AMetDCVG) = RAMetDCVGmol; #(vrisk)
```

```
# Mass balance
          MassBalDCVG = ADCVGIn - ADCVGmol - AMetDCVG; #(vrisk)

#**** Mass Balance for DCVC *****************************************************
# Total production of DCVC
    dt(ADCVCIn) = RAMetDCVGmol * MWDCVC  #(vrisk)
                  + RAMetKid * FracKidDCVC * StochDCVCTCE;#(vrisk)
# Bioactivation of DCVC
    dt(ABioactDCVC) = (kKidBioact * ADCVC);#(vrisk)
# Mass balance
          AUrnNDCVCequiv = AUrnNDCVC/StochN;
          MassBalDCVC = ADCVCIn - ADCVC - ABioactDCVC - AUrnNDCVCequiv;#(vrisk)

#*******************************************************************************
#***                       Dynamic Outputs                                   ***
#*******************************************************************************
# Amount exhaled during exposure (mg)
    dt(AExhExp) = (CInh > 0 ? RAExh : 0);

#*******************************************************************************
#***                       Dose Metrics                                      ***
#*******************************************************************************
#**** AUCs in mg-hr/L unless otherwise noted ***********************************
#AUC of TCE in arterial blood
    dt(AUCCBld) = CArt; #(vrisk)
#AUC of TCE in liver
    dt(AUCCLiv) = CLiv; #(vrisk)
#AUC of TCE in kidney
    dt(AUCCKid) = CKid; #(vrisk)
#AUC of TCE in rapidly perfused
    dt(AUCCRap) = CRap; #(vrisk)
#AUC of TCOH in blood
    dt(AUCCTCOH) = CTCOH; #(vrisk)
#AUC of TCOH in body
    dt(AUCCBodTCOH) = ABodTCOH / VBodTCOH; #(vrisk)
#AUC of free TCA in the plasma (mg/L * hr)
    dt(AUCPlasTCAFree) = CPlasTCAFree; #(vrisk)
#AUC of total TCA in plasma (mg/L * hr)
    dt(AUCPlasTCA) = CPlasTCA; #(vrisk)
#AUC of TCA in liver (mg/L * hr)
    dt(AUCLivTCA) = CLivTCA; #(vrisk)
#AUC of total TCOH (free+gluc) in TCOH-equiv in blood (mg/L * hr)
    dt(AUCTotCTCOH) = CTCOH + CTCOGTCOH; #(vrisk)
#AUC of DCVG in blood (mmol/L * hr) -- NOTE moles, not mg
    dt(AUCCDCVG) = CDCVGmol; #(vrisk)
};
############### End of Dynamics ###################################


CalcOutputs{

#**** Static outputs for comparison to data ***********************************
# TCE
          RetDose = ((InhDose-AExhExp) > 0 ? (InhDose - AExhExp) : 1e-15);
          CAlvPPM = (CAlv < 1.0e-15 ? 1.0e-15 : CAlv * (24450.0 / MWTCE));
          CInhPPM = (ACh< 1.0e-15 ? 1.0e-15 : ACh/VCh*24450.0/MWTCE);
                    # CInhPPM Only used for CC inhalation
          CArt = (CArt < 1.0e-15 ? 1.0e-15 : CArt);
          CVen = (CVen < 1.0e-15 ? 1.0e-15 : CVen);
          CBldMix = (CArt+CVen)/2;
          CFat = (CFat < 1.0e-15 ? 1.0e-15 : CFat);
          CGut = (CGut < 1.0e-15 ? 1.0e-15 : CGut);
          CRap = (CRap < 1.0e-15 ? 1.0e-15 : CRap);
          CSlw = (CSlw < 1.0e-15 ? 1.0e-15 : CSlw);
          CHrt = CRap;
          CKid = (CKid < 1.0e-15 ? 1.0e-15 : CKid);
          CLiv = (CLiv < 1.0e-15 ? 1.0e-15 : CLiv);
          CLung = CRap;
          CMus = (CSlw < 1.0e-15 ? 1.0e-15 : CSlw);
          CSpl = CRap;
          CBrn = CRap;
          zAExh = (AExh < 1.0e-15 ? 1.0e-15 : AExh);
          zAExhpost = ((AExh - AExhExp) < 1.0e-15 ? 1.0e-15 : AExh - AExhExp);
# TCOH
          CTCOH = (CTCOH < 1.0e-15 ? 1.0e-15 : CTCOH);
          CBodTCOH = (ABodTCOH < 1.0e-15 ? 1.0e-15 : ABodTCOH/VBodTCOH);
          CKidTCOH = CBodTCOH;
          CLivTCOH = (ALivTCOH < 1.0e-15 ? 1.0e-15 : ALivTCOH/VLiv);
          CLungTCOH = CBodTCOH;
# TCA
          CPlasTCA = (CPlasTCA < 1.0e-15 ? 1.0e-15 : CPlasTCA);
          CBldTCA = CPlasTCA*TCAPlas;
          CBodTCA = (CBodTCA < 1.0e-15 ? 1.0e-15 : CBodTCA);
          CLivTCA = (CLivTCA < 1.0e-15 ? 1.0e-15 : CLivTCA);
          CKidTCA = CBodTCA;
          CLungTCA = CBodTCA;
          zAUrnTCA = (AUrnTCA < 1.0e-15 ? 1.0e-15 : AUrnTCA);
          zAUrnTCA_sat = (AUrnTCA_sat < 1.0e-15 ? 1.0e-15 : AUrnTCA_sat);
          zAUrnTCA_collect = (AUrnTCA_collect < 1.0e-15 ? 1.0e-15 :
AUrnTCA_collect);
# TCOG
          zABileTCOG = (ABileTCOG < 1.0e-15 ? 1.0e-15 : ABileTCOG);
          #  Concentrations are in TCOH-equivalents
          CTCOG = (CTCOG < 1.0e-15 ? 1.0e-15 : CTCOG);
          CTCOGTCOH = (CTCOG < 1.0e-15 ? 1.0e-15 : StochTCOHGluc*CTCOG);
          CBodTCOGTCOH = (ABodTCOG < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*ABodTCOG/VBodTCOH);
          CKidTCOGTCOH = CBodTCOGTCOH;
          CLivTCOGTCOH = (ALivTCOG < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*ALivTCOG/VLiv);
          CLungTCOGTCOH = CBodTCOGTCOH;
          AUrnTCOGTCOH = (AUrnTCOG < 1.0e-15 ? 1.0e-15 : StochTCOHGluc*AUrnTCOG);
          AUrnTCOGTCOH_sat = (AUrnTCOG_sat < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*AUrnTCOG_sat);
          AUrnTCOGTCOH_collect = (AUrnTCOG_collect < 1.0e-15 ? 1.0e-15 :
StochTCOHGluc*AUrnTCOG_collect);
```

```
# Other
          CDCVGmol = (CDCVGmol < 1.0e-15 ? 1.0e-15 : CDCVGmol);
          CDCVGmol0 = CDCVGmol; #(v1.2.3.2)
          CDCVG_NDtmp = CDFNormal(3*(1-CDCVGmol/CDCVGmolLD));
                    # Assuming LD = 3*sigma_blank, Normally distributed
          CDCVG_ND = ( CDCVG_NDtmp < 1.0 ? ( CDCVG_NDtmp >= 1e-100 ? -
log(CDCVG_NDtmp) : -log(1e-100)) : 1e-100 );
             #(v1.2.3.2)
          zAUrnNDCVC =(AUrnNDCVC < 1.0e-15 ? 1.0e-15 : AUrnNDCVC);
          AUrnTCTotMole = zAUrnTCA / MWTCA + AUrnTCOGTCOH / MWTCOH;
          TotCTCOH = CTCOH + CTCOGTCOH;
          TotCTCOHcomp = CTCOH + CTCOG; # ONLY FOR COMPARISON WITH HACK
          ATCOG = ABodTCOG + ALivTCOG; # ONLY FOR COMPARISON WITH HACK
# Misc
          CVenMole = CVen / MWTCE;
          CPlasTCAMole = (CPlasTCAMole < 1.0e-15 ? 1.0e-15 : CPlasTCAMole);
          CPlasTCAFreeMole = (CPlasTCAFreeMole < 1.0e-15 ? 1.0e-15 :
CPlasTCAFreeMole);

#**** Additional Dose Metrics *************************************************
#

          TotTCAInBW = TotTCAIn/BW;#(vrisk)

# Scaled by BW^3/4
          TotMetabBW34 = TotMetab/BW75;#(vrisk)
          AMetGSHBW34 = AMetGSH/BW75;#(vrisk)
          TotDoseBW34 = TotDose/BW75;#(vrisk)
          AMetLiv1BW34 = AMetLiv1/BW75;#(vrisk)
          TotOxMetabBW34 = (AMetLng+AMetLiv1)/BW75;#(vrisk)
          AMetLngBW34 = AMetLng/BW75; #(vrisk)
          ABioactDCVCBW34 = ABioactDCVC/BW75;#(vrisk)
          AMetLivOtherBW34 = AMetLivOther/BW75; #(vrisk)

# Scaled by tissue volume
          AMetLiv1Liv = AMetLiv1/VLiv; #(vrisk)
          AMetLivOtherLiv = AMetLivOther/VLiv; #(vrisk)
          AMetLngResp = AMetLng/VRespEfftmp; #(vrisk)
          ABioactDCVCKid = ABioactDCVC/VKid;#(vrisk)

#**** Fractional Volumes

          VFatCtmp = VFat/BW; #(vrisk)
          VGutCtmp = VGut/BW; #(vrisk)
          VLivCtmp = VLiv/BW; #(vrisk)
          VRapCtmp = VRap/BW; #(vrisk)
          VRespLumCtmp = VRespLum/BW; #(vrisk)
          VRespEffCtmp = VRespEfftmp/BW; #(vrisk)
          VKidCtmp = VKid/BW; #(vrisk)
          VBldCtmp = VBld/BW; #(vrisk)
          VSlwCtmp = VSlw/BW; #(vrisk)
          VPlasCtmp = VPlas/BW; #(vrisk)
          VBodCtmp = VBod/BW; #(vrisk)
          VBodTCOHCtmp = VBodTCOH/BW; #(vrisk)


};
```

A.8. REFERENCES

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APPENDIX B

Systematic Review of Epidemiologic Studies on Cancer and Trichloroethylene (TCE) Exposure

CONTENTS—Appendix B: Systematic Review of Epidemiologic Studies on Cancer and Trichloroethylene (TCE) Exposure

CONTENTS (continued)

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APPENDIX B. SYSTEMATIC REVIEW OF EPIDEMIOLOGIC STUDIES ON CANCER AND TRICHLOROETHYLENE (TCE) EXPOSURE

B.1. INTRODUCTION

The epidemiologic evidence on trichloroethylene (TCE) is large with over 50 studies identified as of June 2009 and includes occupational cohort studies, case-control studies, both nested within a cohort (nested case-control study) or population based, and geographic based studies. The analysis of epidemiologic studies on cancer and TCE serves to document essential design features, exposure assessment approaches, statistical analyses, and potential sources of confounding and bias. These studies are described below and reviewed according to criteria to assess (1) their ability to inform weight of evidence evaluation for TCE exposure and a cancer hazard and (2) their utility for examination using meta-analysis approaches. A secondary goal of the qualitative review is to provide transparency on study strengths and weaknesses, providing background for inclusion or exclusion of individual studies for quantitative treatment using meta-analysis approaches. Individual study qualities are discussed according to specific criteria in Section B.2.1 to B.2.8., and rationale for studies examined using meta-analysis approaches, the systematic review, contained in Section B.2.9. Appendix C contains a full discussion of the meta-analysis, its analytical methodology, including sensitivity analyses, and findings. This analysis supports discussion of site-specific cancer observations in Chapter 4 where a presentation may be found of study findings with assessment and discussion of observations according to a study's weight of evidence and potential for alternative explanations, including bias and confounding.

B.2. METHODOLOGIC REVIEW OF EPIDEMIOLOGIC STUDIES ON CANCER AND TRICHLOROETHYLENE

Epidemiologic studies considered in this analysis assess the relationship between TCE exposure and cancer, and are identified using several sources and their utility for characterizing hazard and quantitative treatment is based on recommendations in National Research Council (NRC, 2006). A thorough search of the literature was carried out through June 2009 without restriction on year of publication or language using the following approaches: a search of the bibliographic database PubMed (http://www.ncbi.nlm.nih.gov/ pubmed/), TOXNET (http://toxnet.nlm.nih.gov/) and EMBASE (http://www.embase.com/) using the terms "trichloroethylene cancer epidemiology" and ancillary terms, "degreasers," "aircraft, aerospace or aircraft maintenance workers," "metal workers," and "electronic workers," "trichloroethylene and cohort," or, "trichloroethylene and case-control;" bibliographies of reviews of the TCE epidemiologic literature such as those of the Institute of Medicine (IOM, 2003), NRC (2006, 2009) and Scott and Chiu (2006) and, review of bibliographies of individual studies for relevant studies not identified in the previous two approaches. NRC (2006) noted "a full review of the literature should identify all published studies in which there was a possibility that trichloroethylene was investigated, even though results per se may not have been reported."

Additional steps of U.S. Environmental Protection Agency (U.S. EPA) staff to identify studies not published in the literature included contacting primary investigators for case-control studies of liver, kidney and lymphoma and occupation, asking for information on analyses examining trichloroethylene uniquely and a review of Agency for Toxic Substances and Disease Registry (ATSDR) or state health department community health surveys or statistics reviews for information on TCE exposure and cancer incidence or mortality.

The breadth of the available epidemiologic database on trichloroethylene and cancer is wide compared to that available for other chemicals assessed by U.S. EPA. However, few studies were designed with the sole, or primary, objective of this report—to characterize the magnitude of underlying association, if such exists, between TCE and cancer. Yet, many studies in the body of evidence can provide information for identifying cancer hazard and dose-response inferences. The weight a study contributes to the overall evidence on TCE and cancer depends on a number of characteristics regarding the design, exposure assessment, and analysis approaches. Epidemiologic studies were most informative for analysis if they approached ideals described below, as evaluated using objective criteria for identifying a cancer hazard.

Seventy-five studies potentially relevant to health assessment of TCE exposure and cancer and identified from the above comprehensive search are presented in Tables B-1, B-2, and B-3. The studies vary widely in their approaches to study design, exposure assessment, and statistical analysis; for these reasons, studies vary in their usefulness for identifying cancer hazard. Studies are reviewed according to a set of *a priori* guidelines of their utility for assessing TCE exposure and cancer according to the below criteria. Studies approaching criteria ideals contribute greater weight in the weight of evidence analysis than studies with significant deficiencies. These criteria are not meant to be used to "accept" or "reject" a particular study for identifying cancer hazard. Rather, they are to be used as measurement tools for evaluating a study's ability to identify TCE exposure and cancer outcomes. Studies suitable for meta-analysis treatment are selected according to specific criteria identified in B.2.9.4. Individual study descriptions and abstract sheets according to these criteria are found in Section B.3. Appendix C describes meta-analysis methods and findings.

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Aircraft and aerospace workers			
Radican et al. (2008), Blair et al. (1998)	Civilian aircraft-maintenance workers with at least 1 yr in 1952−1956 at Hill Air Force Base, UT. Vital status (VS) to 1990 (Blair et al. 1998) or 2000 (Radican et al., 2008); cancer incidence 1973–1990 (Blair et al., 1998).	14,457 (7,204 ever exposed to TCE). Incidence (Blair et al., 1998) and mortality rates (Blair et al., 1998; Radican et al., 2008) of nonchemical exposed subjects.	Most subjects (*n* = 10,718) with potential exposure to 1 to 25 solvents. Cumulative TCE assigned to individual subjects using JEM. Exposure-response patterns assessed using cumulative exposure, continuous or intermittent exposures, and peak exposure. TCE replaced in 1968 with 1,1,1-trichloroethane and was discontinued in 1978 in vapor degreasing activities. Median TCE exposures were about 10 ppm for rag and bucket; 100−200 ppm for vapor degreasing. Poisson regression analyses controlled for age, calendar time, sex (Blair et al., 1998) or Cox proportional hazard model for age and race.
Krishnadasan et al. (2007)	Nested case-control study within a cohort of 7,618 workers employed for between 1950 and 1992, or who had started employment before 1980 at Boeing/Rockwell/ Rocketdyne (Santa Susana Field Laboratory, [the UCLA cohort of Morgenstern et al., 1997]). Cancer incidence 1988–1999.	326 prostate cancer cases, 1,805 controls. Response rate: Cases, 69%; Controls, 60%.	JEM for TCE, hydrazine, PAHs, benzene, mineral oil constructed from company records, walk-through, or interviews. Lifestyle factors obtained from living subjects through mail and telephone surveys. Conditional logistic regression controlled for cohort, age at diagnosis, physical activity, SES and other occupational exposure (benzene, PAHs, mineral oil, hydrazine).
Zhao et al. (2005); Ritz et al. (1999)	Aerospace workers with >2 yrs of employment at Rockwell/ Rocketdyne (now Boeing) and who worked at Santa Susana Field Laboratory, Ventura, CA, from 1950-1993 (the UCLA cohort of Morgenstern et al. [1997]). Cancer mortality as of December 31, 2001. Cancer incidence 1988–2000 for subjects alive as of 1988.	6,044 (2,689 with high cumulative exposure to TCE). Mortality rates of subjects in lowest TCE exposure category. 5,049 (2,227 with high cumulative exposure to TCE). Incidence rates of subjects in lowest TCE exposure category.	JEM for TCE, hydrazine, PAHs, mineral oil, and benzene. IH ranked each job title ranked for presumptive TCE exposure as high (3), medium (2), low (1), or no (0) exposure for 3 time periods (1951–1969, 1970–1979, 1980–1989). Cumulative TCE score: low (up to 3), medium (over 3 up to 12), high (over 12) assigned to individual subjects using JEM. Cox proportional hazard, controlled for time, since 1st employment, SES, age at diagnosis and hydrazine.

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Boice et al. (2006a)	Aerospace workers with >6 months employment at Rockwell/ Rocketdyne (Santa Susana Field Laboratory and nearby facilities) from 1948–1999 (IEI cohort, IEI [2005]). VS to 1999.	41,351, 1,642 male hourly test stand mechanics (1,111 with potential TCE exposure). Mortality rates of United States population and California population. Internal referent groups including male hourly nonadministrative Rocketdyne workers; male hourly, nonadministrative SSFL workers; and test stand mechanics with no potential exposure to TCE.	Potential TCE exposure assigned to test stands workers only whose tasks included the cleaning or flushing of rocket engines (engine flush) (n = 639) or for general utility cleaning (n = 472); potential for exposure to large quantities of TCE was much greater during engine flush than when TCE used as a utility solvent. JEM for TCE and hydrazine without semiquantitative intensity estimates. Exposure to other solvents not evaluated due to low potential for confounding (few exposed, low exposure intensity, or not carcinogenic). Exposure metrics included employment duration, employment decade, years worked with potential TCE exposure, and years worked with potential TCE exposure via engine cleaning, weighted by number of tests. Lifetable (SMR); Cox proportional hazard controlling for birth year, hire year, and hydrazine exposure.
Boice et al. (1999)	Aircraft-manufacturing workers with at least 1 yr >1960 at Lockheed Martin (Burbank, CA). VS to 1996.	77,965 (2,267 with potential routine TCE exposures and 3,016 with routine or intermittent TCE exposure). Mortality rates of United States population (routine TCE exposed subjects) and non-exposed internal referents (routine and intermittent TCE exposed subjects).	12% with potential routine mixed solvent exposure and 30% with route or intermittent solvent exposure. JEM for potential TCE exposure on (1) routine basis or (2) intermittent or routine basis without semiquantitative intensity estimate. Exposure-response patterns assessed by any exposure or duration of exposure and internal control group. Vapor degreasing with TCE before 1966 and PCE, afterwards. Lifetable analyses (SMR); Poisson regression analysis adjusting for birth date, starting employment date, finishing employment date, sex and race.
Morgan et al. (1998)	Aerospace workers with >6 months 1950–1985 at Hughes (Tucson, AZ). VS to 1993.	20,508 (4,733 with TCE exposures). Mortality rates of United States population for overall TCE exposure; mortality rates of all-other cohort subjects (internal referents) for exposure-response analyses.	TCE exposure intensity assigned using JEM. Exposure-response patterns assessed using cumulative exposure (low versus high) and job with highest TCE exposure rating (peak, medium/high exposure versus no/low exposure). "High exposure" job classification defined as >50 ppm. Vapor degreasing with TCE 1952-1977, but limited IH data <1975. Limited IH data before 1975 and medium/ low rankings likely misclassified given temporal changes in exposure intensity not fully considered (NRC, 2006).

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Costa et al. (1989)	Aircraft manufacturing workers employed 1954–1981at plant in Italy. VS to 1981.	8,626 subjects Mortality rates of the Italian population.	No exposure assessment to TCE and job titles grouped into one of four categories: blue- and white-collar workers, technical staff, and administrative clerks. Lifetable (SMR).
Garabrant et al. (1988)	Aircraft manufacturing workers >4 yrs employment and who had worked at least 1 d at San Diego, CA, plant 1958–1982. VS to 1982.	14,067 Mortality rates of United States population.	TCE exposure assessment for 70 of 14,067 subjects; 14 cases of esophageal cancer and 56 matched controls. For these 70 subjects, company work records identified 37% with job title with potential TCE exposure without quantitative estimates. Lifetable (SMR).
Cohorts Identified From Biological Monitoring (U-TCA)			
Hansen et al. (2001)	Workers biological monitored using U-TCA and air-TCE, 1947–1989. Cancer incidence from 1964–1996.	803 total Cancer incidence rates of the Danish population.	712 with U-TCA, 89 with air-TCE measurement records, 2 with records of both types. U-TCA from 1947−1989; air TCE measurements from 1974. Historic median exposures estimated from the U-TCA concentrations were: 9 ppm for 1947 to 1964, 5 ppm for 1965 to 1973, 4 ppm for 1974 to 1979, and 0.7 ppm for 1980 to 1989. Air TCE measurements from 1974 onward were 19 ppm (mean) and 5 ppm (median). Overall, median TCE exposure to cohort as extrapolated from air TCE and U-TCA measurements was 4 ppm (arithmetic mean, 12 ppm). Exposure metrics: year 1st employed, employment duration, mean exposure, cumulative exposure. Exposure metrics: employment duration, average TCE intensity, cumulative TCE, period 1st employment. Lifetable analysis (SIR).
Anttila et al. (1995)	Workers biological monitored using U-TCA, 1965–1982. VS 1965−1991 and cancer incidence 1967–1992.	3,974 total (3,089 with U-TCA measurements]). Mortality and cancer incidence rates of the Finnish population.	Median U-TCA, 63 µmol/L for females and 48 µmol/L for males; mean U-TCA was 100 µmol/L. Average 2.5 U-TCA measurements per individual. Using the Ikeda et al. (1972) relationship for TCE exposure to U-TCA, TCE exposures were roughly 4 ppm (median) and 6 ppm (mean). Exposure metrics: years since 1st measurement. Lifetable analysis (SMR, SIR).
Axelson et al. (1994)	Workers biological monitored using U-TCA, 1955–1975. VS to 1986 and cancer incidence 1958–1987.	1,4,21 males Mortality and cancer incidence rates of Swedish male population.	Biological monitoring for U-TCA from 1955 and 1975. Roughly ¾ of cohort had U-TCA concentrations equivalent to <20 ppm TCE. Exposure metrics: duration exposure, mean U-TCA. Lifetable analysis (SMR, SIR).

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Other Cohorts			
Clapp and Hoffman (2008)	Deaths between 1969-2001 among employees >5 yrs employment duration at an IBM facility (Endicott, NY).	360 deaths Proportion of deaths among New York residents during 1979 to 1998.	No exposure assessment to TCE. PMR analysis.
Sung et al. (2007, 2008)	Female workers 1st employed 1973-1997 at an electronics (RCA) manufacturing factory (Taoyuan, Taiwan). Cancer incidence 1979–2001 (Sung et al., 2007). Childhood leukemia 1979−2001 among first born of female subjects in Sung et al. (2007, 2008).	63,982 females and 40,647 females with 1st live born offspring. Cancer incidence rates of Taiwan population (Sung et al., 2007). Childhood leukemia incidence rates of first born live births of Taiwan population (Sung et al., 2007).	No exposure assessment. Chlorinated solvents including TCE and PCE found in soil and groundwater at factory site. Company records indicated TCE not used 1975–1991 and PCE 1975–1991 and PCE after 1981. No information for other time periods. Exposure-response using employment duration. Lifetable analysis (SMR, SIR) (Chang et al., 2003, 2005; Sung et al., 2007) or Poisson regression adjusting for maternal age, education, sex, and birth year (Sung et al., 2008).
Chang et al. (2005), Chang et al. (2003)	Male and female workers employed 1978–1997 at electronics factory as studied by Sung et al. (2007). VS from 1985–1997 and cancer incidence 1979–1997.	86,868 total Incidence (Chang et al., 2005) or mortality (Chang et al., 2003) rates Taiwan population.	
ATSDR (2004)	Workers 1952–1980 at the View-Master factory (Beaverton, OR).	616 deaths 1989–2001 Proportion of deaths between 1989−2001 in Oregon population.	No exposure information on individual subjects. TCE and other VOCs detected in well water at the time of the plant closure in 1998 were TCE, 1,220−1,670 µg/L; 1,1-DCE, up to 33 µg/L; and, PCE up to 56 µg/L. PMR analysis.
Raaschou-Nielsen et al. (2003)	Blue-collar workers employed >1968 at 347 Danish TCE-using companies. Cancer incidence through 1997.	40,049 total (14,360 with presumably higher level exposure to TCE). Cancer incidence rates of the Danish population.	Employers had documented TCE usage but no information on individual subjects. Blue-collar versus white-collar workers and companies with <200 workers were variables identified as increasing the likelihood for TCE exposure. Subjects from iron and metal, electronics, painting, printing, chemical, and dry cleaning industries. Median exposures to trichloroethylene were 40−60 ppm for the years before 1970, 10−20 ppm for 1970 to 1979, and approximately 4 ppm for 1980 to 1989. Exposure metrics: employment duration, year 1st employed, and # employees in company. Lifetable (SIR).

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Ritz (1999a)	Male uranium-processing plant workers >3 months employment 1951–1972 at DOE facility (Fernald, OH). VS 1951–1989, cancer.	3,814 white males monitored for radiation (2,971 with potential TCE exposure). Mortality rates of the United States population; Non-TCE exposed internal controls for TCE exposure-response analyses.	JEM for TCE, cutting fluids, kerosene, and radiation generated by employees and industrial hygienists. Subjects assigned potential TCE according to intensity: light (2,792 subjects), moderate (179 subjects), heavy (no subjects). Lifetable (SMR) and conditional logistic regression adjusted for pay status, date first hire, radiation.
Henschler et al. (1995)	Male workers > 1 yr 1956–1975 at cardboard factory (Arnsberg region, Germany). VS to 1992.	169 exposed; 190 unexposed Mortality rates from German Democratic Republic (broad categories) or renal cell carcinoma incidence rates from Danish population, German Democratic, or non-TCE exposed subjects.	Walk-through surveys and employee interviews used to identify work areas with TCE exposure. TCE exposure assigned to renal cancer cases using workman's compensation files. Lifetable (SMR, SIR) or Mantel-Haenszel.
Greenland et al. (1994)	Cancer deaths, 1969–1984, among pensioned workers employed <1984 at GE transformer manufacturing plant (Pittsfield, MA), and who had job history record; controls were noncancer deaths among pensioned workers.	512 cases, 1,202 controls. Response rate: Cases, 69%; Controls, 60%.	Industrial hygienist assessment from interviews and position descriptions. TCE (no/any exposure) assigned to individual subjects using JEM. Logistic regression.
Sinks et al. (1992)	Workers employed 1957–1980 at a paperboard container manufacturing and printing plant (Newnan, GA). VS to 1988. Kidney and bladder cancer incidence through 1990.	2,050 total Mortality rates of the United States population, bladder and kidney cancer incidence rates from the Atlanta-SEER registry for the years 1973−1977.	No exposure assessment to TCE; analyses of all plant employees including white- and blue-collar employees. Assignment of work department in case-control study based upon work history; Material Safety Data Sheets identified chemical usage by department. Lifetable (SMR, SIR) or conditional logistic regression adjusted for hire date and age at hire, and using 5- and 10-year lagged employment duration.

Table B-1. Description of epidemiologic cohort and PMR studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (N) Comparison group (N)	Exposure assessment and other information
Blair et al. (1989)	Workers employed 1942- 1970 in U.S. Coast. VS to 1980.	3,781 males of whom 1,767 were marine inspectors (48%). Mortality rates of the United States population. Mortality rates of marine inspectors also compared to that of noninspectors.	No exposure assessment to TCE. Marine inspectors worked in confined spaces and had exposure potential to multiple chemicals. TCE was identified as one of 10 potential chemical exposures. Lifetable (SMR) and directly adjusted relative risks.
Shannon et al. (1988)	Workers employed ≥6 months at GE lamp manufacturing plant, 1960-1975. Cancer incidence from 1964-1982.	1,870 males and females, 249 (13%) in coiling and wire-drawing area. Cancer incidence rates from Ontario Cancer Registry.	No exposure assessment to TCE. Workers in coiling and wire drawing (CWD) had potential exposure to many chemicals including metals and solvents. A 1955-dated engineering instruction sheet identified trichloroethylene used as degreasing solvent in CWD. Lifetable (SMR).
Shindell and Ulrich (1985)	Workers employed >3 months at a TCE manufacturing plant 1957–1983. VS to 1983.	2,646 males and females Mortality rates of the United States population.	No exposure assessment to TCE; job titles categorized as either white- or blue-collar. Lifetable analysis (SMR).
Wilcosky et al. (1984)	Respiratory, stomach, prostate, lymphosarcoma, and lymphatic leukemia cancer deaths 1964–1972 among 6,678 active and retired production workers at a rubber plant (Akron, OH); controls were a 20% age-stratified random sample of the cohort.	183 cases (101 respiratory, 33 prostate, 30 stomach, 9 lymphosarcoma and 10 lymphatic leukemia cancer deaths).	JEM without quantitative intensity estimates for 20 exposures including TCE. Exposure metric: ever held job with potential TCE exposure.

DCE = dichloroethylene, DOE = U.S. Department of Energy, IEI = International Epidemiology Institute, JEM = job-exposure matrix, NRC = National Research Council, PCE = perchloroethylene, PMR = proportionate mortality ratio, SIR = standardized incidence ratio, SMR = standardized mortality ratio, SSFL = Santa Susanna Field Laboratory, U-TCA = urinary trichloroacetic acid, UCLA = University of California, Los Angeles, VOCs = volatile organic compounds, VS = vital status.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Bladder			
Pesch et al. (2000a)	Histologically confirmed urothelial cancer (bladder, ureter, renal pelvis) cases from German hospitals (5 regions) in 1991−1995; controls randomly selected from residency registries matched on region, sex, and age.	1,035 cases 4,298 controls Cases, 84%; Controls, 71%	Occupational history using job title or self-reported exposure. JEM and JTEM to assign exposure potential to metals and solvents (chlorinated solvents, TCE, PCE). Lifetime exposure to TCE exposure examined as 30th, 60th, and 90th percentiles (medium, high, and substantial) of exposed control exposure index. Duration used to examine occupational title and job task duties and defined as 30th, 60th, and 90th percentiles (medium, long, and very long) of exposed control durations. Logistic regression with covariates for age, study center, and smoking.
Siemiatycki et al. (1994), Siemiatycki (1991)	Male bladder cancer cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing (RDD).	484 cases 533 population controls; 740 other cancer controls Cases, 78%; Controls, 72%	JEM to assign 294 exposures including TCE on semiquantitative scales categorized as any or substantial exposure. Other exposure metrics included exposure duration in occupation or job title. Logistic regression adjusted for age, ethnic origin, socioeconomic status, smoking, coffee consumption, and respondent status [occupation or job title] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
Brain			
De Roos et al. (2001) Olshan et al. (1999)	Neuroblastoma cases in children of <19 yrs selected from Children's Cancer Group and Pediatric Oncology Group with diagnosis in 1992−1994; population controls (RDD) matched to control on birth date.	504 cases 504 controls Cases, 73%; Controls, 74%	Telephone interview with parent using questionnaire to assess parental occupation and self-reported exposure history and judgment-based attribution of exposure to chemical classes (halogenated solvents) and specific solvents (TCE). Exposure metric was any potential exposure. Logistic regression with covariate for child's age and material race, age, and education.
Heineman et al. (1994)	White, male cases, age >30 yrs, identified from death certificates in 1978−1981; controls identified from death certificates and matched for age, year of death and study area.	300 cases 386 controls Cases, 74%; Controls, 63%	In-person interview with next-of-kin; questionnaire assessing lifetime occupational history using job title and JEM of Gomez et al. (1994). Cumulative exposure metric (low, medium or and high) based on weighted probability and duration. Logistic regression with covariates for age and study area.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Colon and Rectum			
Goldberg et al. (2001), Siemiatycki (1991)	Male colon cancer cases, 35−75 yrs, from 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing (RDD).	497 cases 533 population controls and 740 cancer controls Cases, 82%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, ethnic origin, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source socioeconomic status, smoking, coffee consumption, and respondent status [occupation, some chemical agents] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status [TCE].
Dumas et al. (2000), Simeiatycki (1991)	Male rectal cancer cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	292 cases 533 population controls and 740 other cancer controls Cases, 78%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and body mass index [TCE] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption [TCE].
Fredriksson et al. (1989)	Colon cancer cases aged 30−75 yrs identified through the Swedish Cancer Registry among patients diagnosed in 1980−1983; population-based controls were frequency-matched on age and sex and were randomly selected from a population register.	329 cases 658 controls Not available	Mailed questionnaire assessing occupational history with telephone interview follow-up. Self-reported exposure to TCE defined as any exposure. Mantel-Haenszel stratified on age, sex, and physical activity.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Esophagus			
Parent et al. (2000a), Siemiatycki (1991)	Male esophageal cancer cases, 35−75 yrs, diagnosed in 19 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	292 cases 533 population controls; 740 subjects with other cancers Cases, 78%; controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and body mass index [solvents] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption [TCE].
Lymphoma			
Wang et al. (2009)	Cases among females aged 21 and 84 yrs with NHL in 1996−2000 and identified from Connecticut Cancer Registry; population-based female controls (1) if <65 yrs of age, having Connecticut address stratified by 5-yr age groups identified from random digit dialing or (2) >65 yrs of age, by random selection from Centers for Medicare and Medicaid Service files.	601 cases 717 controls Cases, 72%; Controls, 69% (<65 yrs), 47% (>65 yrs)	In-person interview with using questionnaire assessment specific jobs held for >1 yr. Intensity and probability of exposure to broad category of organic solvents and to individual solvents, including TCE, estimated using JEM (Gomez et al, 1994; Dosemeci et al., 1994) and assigned blinded. Exposure metric of any exposure, exposure intensity (low, medium/high), and exposure probability (low, medium/high). Logistic regression adjusted for age, family history of hematopoietic cancer, alcohol consumption and race.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Constantini et al. (2008), Miligi et al. (2006)	Cases aged 20−74 with NHL, including CLL, all forms of leukemia, or multiple myeloma (MM) in 1991−1993 and identified through surveys of hospital and pathology departments in study areas and in specialized hematology centers in 8 areas in Italy; population-based controls stratified by 5-yr age groups and by sex selected through random sampling of demographic or of National Health Service files.	1,428 NHL + CLL, 586 Leukemia, 263, MM 1,278 controls (leukemia analysis) 1,100 controls (MM analysis) Cases, 83%; Controls, 73%	In-person interview primarily at interviewee's home (not blinded) using questionnaire assessing specific jobs, extra occupational exposure to solvents and pesticides, residential history, and medical history. Occupational exposure assessed by job-specific or industry-specific questionnaires. JEM used to assign TCE exposure and assessed using intensity (2 categories) and exposure duration (2 categories). All NHL diagnoses and 20% sample of all cases confirmed by panel of 3 pathologists. Logistic regression with covariates for sex, age, region, and education. Logistic regression for specific NHL included an additional covariate for smoking.
Seidler et al. (2007) Mester et al. (2006) Becker et al. (2004)	NHL and Hodgkin's disease cases aged 18−80 yrs identified through all hospitals and ambulatory physicians in six regions of Germany between 1998 and 2003; population controls were identified from population registers and matched on age, sex, and region.	710 cases 710 controls Cases, 87%; Controls, 44%	In-person interview using questionnaire assessing personal characteristics, lifestyle, medical history, UV light exposure, and occupational history of all jobs held for >1 yr. Exposure of a priori interest were assessed using job task-specific supplementary questionnaires. JEM used to assign cumulative quantitative TCE exposure metric, categorized according to the distribution among the control persons (50th and 90th percentile of the exposed controls). Conditional logistic regression adjusted for age, sex, region, smoking and alcohol consumption.
Persson and Fredriksson (1999) Combined analysis of NHL cases in Persson et al. (1993), Persson et al. (1989)	Histologically confirmed cases of B-cell NHL, age 20−79 yrs, identified in two hospitals in Sweden: Oreboro in 1964−1986 (Persson et al., 1989) and in Linkoping between 1975−1984 (Persson et al., 1993); controls were identified from previous studies and were randomly selected from population registers.	NHL cases, 199 479 controls Cases, 96% (Oreboro), 90% (Linkoping); controls, not reported	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Unadjusted Mantel-Haenszel chi-square.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Nordstrom et al. (1998)	Histologically-confirmed cases in males of hairy-cell leukemia reported to Swedish Cancer Registry in 1987−1992 (includes one case latter identified with an incorrect diagnosis date); population-based controls identified from the National Population Registry and matched (1:4 ratio) to cases for age and county.	111 cases 400 controls Cases, 91%; Controls, 83%	Mailed questionnaire to assess self reported working history, specific exposure, and leisure time activities. Univariate analysis for chemical-specific exposures (any TCE exposure).
Fritschi and Siemiatycki, 1996a), Siemiatycki (1991)	Male NHL cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	215 cases 533 population controls (Group 1) and 1,900 subjects with other cancers (Group 2) Cases, 83%; Controls, 71%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Exposure metric defined as any or substantial exposure. Logistic regression adjusted for age, proxy status, income, and ethnicity [solvents] or Mantel-Haenszel stratified by age, body mass index, and cigarette smoking [TCE].
Hardell et al. (1994, 1981)	Histologically-confirmed cases of NHL in males, age 25−85 yrs, admitted to Swedish (Umea) hospital between 1974−1978; living controls (1:2 ratio) from the National Population Register, matched to living cases on sex, age, and place of residence; deceased controls from the National Registry for Causes of Death, matched (1:2 ratio) to dead cases on sex, age, place of residence, and year of death.	105 cases 335 controls Response rate not available	Self-administered questionnaire assessing self-reported solvent exposure; phone follow-up with subject, if necessary. Unadjusted Mantel-Haenszel chi-square.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Persson et al. (1993), Persson et al. (1989)	Histologically confirmed cases of Hodgkin's disease, age 20−80 yrs, identified in two hospitals in Sweden: Oreboro in 1964−1986 (Persson et al., 1989) and in Linkoping between 1975−1984 (Persson et al., 1993); controls randomly selected from population registers.	54 cases (1989 study); 31 cases (1993 study) 275 controls (1989 study); 204 controls (1993 study) Response rate not available	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Logistic regression with adjustment for age and other exposure; unadjusted Mantel-Haenszel chi-square.
Childhood Leukemia			
Shu et al. (2004, 1999)	Childhood leukemia cases, <15 yrs, diagnosed between 1989 and 1993 by a Children's Cancer Group member or affiliated institute; population controls (random digit dialing), matched for age, race, and telephone area code and exchange.	1,842 cases 1,986 controls Cases, 92%; controls, 77%	Telephone interview with mother, and whenever available, fathers using questionnaire to assess occupation using job-industry title and self-reported exposure history. Questionnaire included questions specific for solvent, degreaser or cleaning agent exposures. Logistic regression with adjustment for maternal or paternal education, race, and family income. Analyses of paternal exposure also included age and sex of the index child.
Costas et al. (2002), MA DPH (1997)	Childhood leukemia (<19 yrs age) diagnosed in 1969−1989 and who were resident of Woburn. MA; controls randomly selected from Woburn public School records, matched for age.	19 cases 37 controls Cases, 91%; Controls, not available	Questionnaire administered to parents separately assessing demographic and lifestyle characteristics, medical history information, environmental and occupational exposure and use of public drinking water in the home. Hydraulic mixing model used to infer delivery of TCE and other solvents water to residence. Logistic regression with composite covariate, a weighted variable of individual covariates.
McKinney et al. (1991)	Incident childhood leukemia and non-Hodgkin's lymphoma cases, 1974–1988, ages not identified, from three geographical areas in England; controls randomly selected from children of residents in the three areas and matched for sex and birth health district.	109 cases 206 controls Cases, 72%; Controls, 77%	In-person interview with questionnaire with mother to assess maternal occupational exposure history, and with father and mother, as surrogate, to assess paternal occupational exposure history. No information provided in paper whether interviewer was blinded as to case and control status. Matched pair design using logistic regression for univariate and multivariate analysis.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Lowengart et al. (1987)	Childhood leukemia cases aged <10 yrs and identified from the Los Angeles (CA) Cancer Surveillance Program in 1980−1984; controls selected from RDD or from friends of cases and matched on age, sex, and race.	123 cases 123 controls Cases, 79%; Controls, not available	Telephone interview with questionnaire to assess parental occupational and self-reported exposure history. Matched (discordant) pair analysis.
Melanoma			
Fritschi and Siemiatycki (1996b), Siemiatycki (1991)	Male melanoma cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	103 cases 533 population controls and 533 other cancer controls Cases, 78%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, and ethic origin [TCE] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, and ethnic origin [TCE].
Prostate			
Aronson et al. (1996), Siemiatycki (1991)	Male prostate cancer cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	449 cases 533 population controls (Group 1) and other cancer cases from same study (Group 2) Cases, 81%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Logistic regression adjusted for age, ethnic origin, socioeconomic status, Quetlet, and respondent status [occupation] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status [TCE].

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Renal Cell			
Charbotel et al. (2006, 2009)	Cases from Arve Valley region in France identified from local urologists files and from area teaching hospitals; age- and sex-matched controls chosen from file of same urologist as who treated case or recruited among the patients of the case's general practitioner.	87 cases 316 controls Cases, 74%; controls, 78%	Telephone interview with case or control, or, if deceased, with next-of-kin (22% cases, 2% controls). Questionnaire assessing occupational history, particularly, employment in the screw cutting jobs, and medical history. Semiquantitative TCE exposure assigned to subjects using a task/TCE-Exposure Matrix designed using information obtained from questionnaires and routine atmospheric monitoring of work shops or biological monitoring (U-TCA) of workers carried out since the 1960s. Cumulative exposure, cumulative exposure with peaks, and TWA. Conditional logistic regression with covariates for tobacco smoking and body mass index.
Brüning et al. (2003)	Histologically-confirmed cases 1992−2000 from German hospitals (Arnsberg); hospital controls (urology department) serving area, and local geriatric department, for older controls, matched by sex and age.	134 cases 401 controls Cases, 83%; Controls, not available	In-person interviews with case or next-of-kin; questionnaire assessing occupational history using job title. Exposure metrics included longest job held, JEM of Pannett et al. (1985) to assign cumulative exposure to TCE and PCE, and exposure duration. Logistic regression with covariates for age, sex, and smoking.
Pesch et al. (2000b)	Histologically-confirmed cases from German hospitals (5 regions) in 1991−1995; controls randomly selected from residency registries matched on region, sex, and age.	935 cases 4,298 controls Cases, 88%; Controls, 71%	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title (JEM approach), self-reported exposure, or job task (JTEM approach) to assign TCE and other exposures. Logistic regression with covariates for age, study center, and smoking.
Parent et al. (2000b), Siemiatycki (1991)	Male renal cell carcinoma cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	142 cases 533 population controls (Group 1) and other cancer controls (excluding lung and bladder cancers) (Group 2) Cases, 82%; Controls, 71%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (about 300 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified by age, body mass index, and cigarette smoking [TCE] or logistic regression adjusted for respondent status, age, smoking, and body mass index [occupation, job title].

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Dosemeci et al. (1999)	Histologically-confirmed cases, 1988−1990, white males and females, 20−85 yrs, from Minnesota Cancer Registry; controls stratified for age and sex using RDD, 21−64 yrs, or from HCFA records, 64−85 yrs.	438 cases 687 controls Cases, 87%; Controls, 86%	In-person interviews with case or next-of-kin; questionnaire assessing occupational history of TCE using job title and JEM of Gomez et al. (1994). Exposure metric was any TCE exposure. Logistic regression with covariates for age, smoking, hypertension, and body mass index.
Vamvakas et al. (1998)	Cases who underwent nephrectomy in 1987−1992 in a hospital in Arnsberg region of Germany; controls selected accident wards from nearby hospital in 1992.	58 cases 84 controls Cases, 83%; Controls, 75%	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title or self-reported exposure to assign TCE and PCE exposure. Logistic regression with covariates for age, smoking, body mass index, hypertension, and diuretic intake.
Multiple or Other Sites			
Lee et al. (2003)	Liver, lung, stomach, colorectal cancer deaths in males and females between 1966−1997 from two villages in Taiwan; controls were cardiovascular and cerebral-vascular disease deaths from same underlying area as cases.	53 liver, 39 stomach, 26 colorectal, 41 lung cancer cases 286 controls Response rate not reported	Residence as recorded on death certificate. Mantel-Haenszel stratified by age, sex, and time period.
Kernan et al. (1999)	Pancreatic deaths, 1984-1993, in 24 states; non-cancer death and non-pancreatic disease death controls, frequency matched to cases by age, gender, race and state.	63,097 pancreatic cancer cases 252,386 non-cancer population controls Response rate not reported	Usual occupation and industry on death certificate coded to standardized occupation codes and industry codes for 1980 U. S. census. Potential exposure to 11 chlorinated hydrocarbons, including TCE, assessed using job-exposure matrix of Gomez et al. (1994). Logistic regression adjusted for age, marital status, gender, race, and metropolitan and residential status.

Table B-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (N) Comparison group (N) Response rates	Exposure assessment and other information
Siemiatycki (1991)	Male cancer cases, 1979−1985, 35−75 yrs, diagnosed in 16 Montreal-area hospitals, histologically confirmed; cancer controls identified concurrently; age-matched, population-based controls identified from electoral lists and RDD.	857 lung and 117 pancreatic cancer cases 533 population controls (Group 1) and other cancer cases from same study (Group 2) Cases, 79% (lung), 71% (pancreas); Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (lung cancer) and age, income, index for cigarette smoking, and respondent status (pancreatic cancer).

HCFA = Health Care Financing Administration, JEM = job-exposure matrix, JTEM = job-task-exposure matrix, NCI = National Cancer Institute, PCE = perchloroethylene, RDD = random digit dialing, U-TCA = urinary trichloroacetic acid, UV = ultra-violet.

Table B-3. Geographic-based studies assessing cancer and TCE exposure

Reference	Description	Analysis approach	Exposure assessment
Broome County, NY Studies			
ATSDR (2006a, 2008)	Total, 22 site-specific, and childhood cancer incidence from 1980−2001 among residents in 2 areas in Endicott, NY.	SIR among all subjects (ATSDR, 2006a) or among white subjects only (ATSDR, 2008) with expected numbers of cancers derived using age-specific cancer incidence rates for New York State, excluding New York City. Limited assessment of smoking and occupation using medical and other records in lung and kidney cancer subjects (ATSDR, 2008).	Two study areas, Eastern and Western study areas, identified based on potential for soil vapor intrusion exposures as defined by the extent of likely soil vapor contamination. Contour lines of modeled VOC soil vapor contamination levels based on exposure model using GIS mapping and soil vapor sampling results taken in 2003. The study areas were defined by 2000 Census block boundaries to conform to model predicted areas of soil vapor contamination. TCE was the most commonly found contaminant in indoor air in Eastern study area at levels ranging from 0.18 to 140 μg/m3 , with tetrachloroethylene, cis-1,2-dichloroethene, 1,1,1-trichloroethane, 1,1-dichloroethylene, 1,1-dichloroethane, and Freon 113 detected at lower levels. PCE was most common contaminant in indoor air in Western study area with other VOCs detected at lower levels.
Maricopa County, AZ Studies			
Aickin et al. (1992) Aickin (2004)	Cancer deaths, including leukemia, 1966−1986, and childhood (<19 yrs old) leukemia incident cases (1965−1986), Maricopa County, AZ.	Standardized mortality RR from Poisson regression modeling. Childhood leukemia incidence data evaluated using Bayes methods and Poisson regression modeling.	Location of residency in Maricopa County, AZ, at the time of death as surrogate for exposure. Some analyses examined residency in West Central Phoenix and cancer. Exposure information is limited to TCE concentration in two drinking water wells in 1982.
Pima County, AZ Studies			
AZ DHS (1990, 1995)	Cancer incidence in children (<19 yrs old) and testicular cancer in 1970−1986 and 1987−1991, Pima County, AZ.	Standardized incidence RR from Poisson regression modeling using method of Aickin et al. (1992). Analysis compares incidence in Tucson Airport Area to rate for rest of Pima County.	Location of residency in Pima, County, AZ, at the time of diagnosis or death as surrogate for exposure. Exposure information is limited to monitoring since 1981 and includes VOCs in soil gas samples (TCE, PCE, 1,1-dichloroethylene, 1,1,1-trichloroacetic acid); PCBs in soil samples, and TCE in municipal water supply wells.
Other			
Coyle et al. (2005)	Incident breast cancer cases among men and women, 1995-2000, reported to Texas Cancer Registry	Correlation study using rank order statistics of mean average annual breast cancer rate among women and men and atmospheric release of 12 hazardous air pollutants.	Reporting to EPA Toxic Release Inventory the number of pounds released for 12 hazardous air pollutants, (carbon tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, trichloroethylene, arsenic, cadmium, chromium, cobalt, copper, and nickel).

Table B-3. Geographic-based studies assessing cancer and TCE exposure (continued)

Reference	Description	Analysis approach	Exposure assessment
Morgan and Cassady (2002)	Incident cancer cases, 1988–1989, among residents of 13 census tracts in Redlands area, San Bernardino County, CA.	SIR for all cancer sites and 16 site-specific cancers; expected numbers using incidence rates of site-specific cancer of a four-county region between 1988−1992.	TCE and perchlorate detected in some county wells; no information on location of wells to residents, distribution of contaminated water, or TCE exposure potential to individual residents in studied census tracts.
Vartiainen et al. (1993)	Total cancer and site-specific cancer cases (lymphoma sites and liver) from 1953−1991 in two Finnish municipalities.	SIR with expected number of cancers and site-specific cancers derived from incidence of the Finnish population.	Monitoring data from 1992 indicated presence of TCE, tetrachloroethylene and 1,1,1,-trichloroethane in drinking water supplies in largest towns in municipalities. Residence in town used to infer exposure to TCE.
Cohn et al. (1994) Fagliano et al. (1990)	Incident leukemia and NHL cases, 1979−1987, from 75 municipalities and identified from the New Jersey State Cancer Registry. Histological type classified using WHO scheme and the classification of NIH Working Formulation Group for grading NHL.	Logistic regression modeling adjusted for age.	Monitoring data from 1984−1985 on TCE, THM, and VOCs concentrations in public water supplies, and historical monitoring data conducted in 1978−1984.
Mallin (1990)	Incident bladder cancer cases and deaths, 1978−1985, among residents of 9 NW Illinois counties.	SIR and SMR by county of residence and zip code; expected numbers of bladder cancers using age-race-sex specific incidence rates from SEER or bladder cancer mortality rates of the United States population from 1978−1985.	Exposure data are lacking for the study population with the exception of noting one of two zip code areas with observed elevated bladder cancer rates also had groundwater supplies contaminated with TCE, PCE and other solvents.

Table B-3. Geographic-based studies assessing cancer and TCE exposure (continued)

Reference	Description	Analysis approach	Exposure assessment
Isacson et al. (1985)	Incident bladder, breast, prostate, colon, lung and rectal cancer cases reported to Iowa cancer registry between 1969−1981.	Age-adjusted site-specific cancer incidence in Iowa towns with populations of 1,000−10,000 and who were serviced by a public drinking water supply.	Monitoring data of drinking water at treatment plant in each Iowa municipality with populations of 1,000−10,000 used to infer TCE and other volatile organic compound concentrations in finished drinking water supplies.

GIS = geographic information system, NW = Northwestern, PCE = perchloroethylene, RR = rate ratio, SEER = Surveillance, Epidemiology, and End Results, SIR = standardized incidence ratio, SMR = standardized mortality ratio, VOCs = volatile organic compounds, WHO = World Health Organization.

Category A: Study Design

- Clear articulation of study objectives or hypothesis. The ideal is a clearly stated hypothesis or study objectives and the study is designed to achieve the identified objectives.
- Selection and characterization in cohort studies of exposure and control groups and of cases and controls (case-control studies) is adequate. The ideal is for selection of cohort and referents from the same underlying population and differences between these groups are due to TCE exposure or level of TCE exposure and not to physiological, health status, or lifestyle factors. Controls or referents are assumed to lack or to have background exposure to TCE. These factors may lead to a downward bias including one of which is known as "healthy worker bias," often introduced in analyses when mortality or incidence rates from a large population such as the U.S. population are used to derive expected numbers of events. The ideal in case-control studies is cases and controls are derived from the same population and are representative of all cases and controls in that population. Any differences between controls and cases are due to exposure to TCE itself and not to confounding factors related to both TCE exposure and disease. Additionally, the ideal is for controls to be free of any disease related to TCE exposure. In this latter case, potential bias is toward the null hypothesis.

Category B: Endpoint Measured

- Levels of health outcome assessed. Three levels of health outcomes are considered in assessing the human health risks associated with exposure to TCE: biomarkers of effects and susceptibility, morbidity, and mortality. Both morbidity as enumerated by incidence and mortality as identified from death certificates are useful indicators in risk assessment for hazard identification. The ideal is for accurate and predictive indicator of disease. Incidence rates are generally considered to provide an accurate indication of disease in a population and cancer incidence is generally enumerated with a high degree of accuracy in cancer registries. Death certifications are readily available and have complete national coverage but diagnostic accuracy is reduced and can vary by specific diagnosis. Furthermore, diagnostic inaccuracies can contribute to death certificates as a poor surrogate for disease incidence. Incidence, when obtained from population-based cancer registries, is preferred for identifying cancer hazards.
- Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma. Classification of lymphomas today is based on morphologic, immunophenotypic, genotypic, and clinical features and is based upon the World Health Organization (WHO) classification, introduced in 2001, and incorporation of WHO terminology into International Classification of Disease (ICD)-0-3. ICD Versions 7 and earlier had rubrics for general types of lymphatic and hematopoietic cancer, but no categories for distinguishing specific types of cancers, such as acute leukemia. Epidemiologic studies based on causes of deaths as coded using these older ICD classifications typically grouped together lymphatic neoplasms instead of examining

individual types of cancer or specific cell types. Before the use of immunophenotyping, these grouping of ambiguous diseases such as non-Hodgkin's lymphoma and Hodgkin's lymphoma may be have misclassified. Lymphatic tumors coding, starting in 1994 with the introduction of the Revised European-American Lymphoma classification, the basis of the current WHO classification, was more similar to that presently used. Misclassification of specific types of cancer, if unrelated to exposure, would have attenuated estimate of relative risk and reduced statistical power to detect associations. When the outcome was mortality, rather than incidence, misclassification would be greater because of the errors in the coding of underlying causes of death on death certificates (IOM, 2003). Older studies that combined all lymphatic and hematopoietic neoplasms must be interpreted with care.

Category C: TCE-Exposure Criteria

- Adequate characterization of exposure. The ideal is for TCE exposure potential known for each subject and quantitative assessment (job-exposure-matrix approach) of TCE exposure assessment for each subject as a function of job title, year exposed, duration, and intensity. Consideration of job task as additional information supplementing job title strengthens assessment increases specificity of TCE assignment. The assessment approach is accurate for assigning TCE intensity (TCE concentration or a time-weighted average) to individual study subjects and estimates of TCE intensity are validated using monitoring data from the time period. The objective for cohort and case-controls studies is to differentiate TCE exposed subjects from subjects with little or no TCE exposure. A variety of dose metrics may be used to quantify or classify exposures for an epidemiologic study. They include precise summaries of quantitative exposure, concentrations of biomarkers, cumulative exposure, and simple qualitative assessments of whether exposure occurred (yes or no). Each method has implicit assumptions and potential problems that may lead to misclassification. Exposure assessment approaches in which it was unclear that the study population was actually exposed to TCE are considered inferior since there may be a lower likelihood or degree of exposure to study subjects compared to approaches which assign known TCE exposure potential to each subject.

Category D: Follow-up (Cohort)

- Loss to follow-up. The ideal is complete follow-up of all subjects; however, this is not achievable in practice, but it seems reasonable to expect loss to follow-up not to exceed 10%. The bias from loss to follow-up is indeterminate. Random loss may have less effect than if subjects who are not followed have some significant characteristics in common.
- Follow-up period allows full latency period for over 50% of the cohort. The ideal to follow all study subjects until death. Short of the ideal, a sufficient follow-up period to

allow for cancer induction period or latency over 15 or 20 years is desired for a large percentage of cohort subjects.

Category E: Interview Type (Case-control)

- Interview approach. The ideal interviewing technique is face-to-face by trained interviewers with more than 90% of interviews with cases and control subjects conduced face-to-face. The effect on the quality of information from other types of data collection is unclear, but telephone interviews and mail-in questionnaires probably increase the rate of misclassification of subject information. The bias is toward the null hypothesis if the proportion of interview by type is the same for case and control, and of indeterminate direction otherwise.
- Blinded interviewer. The ideal is for the interviewer to be unaware whether the subject is among the cases or controls and the subject to be unaware of the purpose and intended use of the information collected. Blinding of the interviewer is generally not possible in a face-to-face interview. In face-to-face and telephone interviews, potential bias may arise from the interviewer expects regarding the relationship between exposure and cancer incidence. The potential for bias from face-to-face interviews is probably less than with mail-in interviews. Some studies have assigned exposure status in a blinded manner using a job-exposure matrix and information collected in the unblinded interview. The potential for bias in this situation is probably less with this approach than for nonblinded assignment of exposure status.

Category F: Proxy Respondents

- Proxy respondents. The ideal is for data to be supplied by the subject because the subject generally would be expected to be the most reliable source; less than 10% of either total cases or total controls for case-control studies. A subject may be either deceased or too ill to participate, however, making the use of proxy responses unavoidable if those subjects are to be included in the study. The direction and magnitude of bias from use of proxies is unclear, and may be inconsistent across studies.

Category G: Sample Size

- The ideal is for the sample size is large enough to provide sufficient statistical power to ensure that any elevation of effect in the exposure group, if present, would be found, and to ensure that the confidence bounds placed on relative risk estimates can be well-characterized.

Category H: Analysis Issues

- Control for potentially confounding factors of importance in analysis. The ideal in cohort studies is to derive expected numbers of cases based on age-sex- and time-specific cancer rates in the referent population and in case-control studies by matching on age and sex in the design and then adjusting for age in the analysis of data. Age and sex are likely correlated with exposure and are also risk factors for cancer development. Similarly, other factors such as cigarette smoking and alcohol consumption are risk factors for several site-specific cancers reported as associative with TCE exposure. To be a confounder of TCE, exposure to the other factor must be correlated, and the association of the factor with the site-specific cancer must be causal. The expected effect from controlling for confounders is to move the estimated relative risk estimate closer to the true value.
- Statistical methods are appropriate. The ideal is that conclusions are drawn from the application of statistical methods that are appropriate to the problem and accurately interpreted.
- Evaluation of exposure-response. The ideal is an examination of a linear exposure-response as assessed with a quantitative exposure metric such as cumulative exposure. Some studies, absent quantitative exposure metrics, examine exposure response relationships using a semiquantitative exposure metric or by duration of exposure. A positive dose-response relationship is usually more convincing of an association as causal than a simple excess of disease using TCE dose metric. However, a number of reasons have been identified for a lack of linear exposure-response finding and the failure to find such a relationship means little from an etiological viewpoint and does not minimize an observed association with overall TCE exposure.
- Documentation of results. The ideal is for analysis observations to be completely and clearly documented and discussed in the published paper, or provided in supplementary materials accompanying publication.

B.2.1. Study Designs and Characteristics

The epidemiologic designs investigating TCE exposure and cancer include cohort studies of occupationally exposure populations, population case-control studies, and geographic studies of residents in communities with TCE in water supplies or ambient air. Analytical epidemiologic studies, which include case-control and cohort designs, are generally relied on for identifying a causal association between human exposure and adverse health effects (U.S. EPA, 2005) due to their clear ability to show exposure precedes disease occurrence. In contrast, ecologic studies such as health surveys of cancer incidence or mortality in a community during a specified time period, i.e., geographic-based studies identified in Appendix B, Table B-3, provide correlations between rates of cancer and exposure measured at the geographic level.

An epidemiologic study's ability to inform a question on TCE and cancer depends on clear articulation of study objective or hypothesis and adequate selection of exposed and control group in cohort studies and cases and controls in case-control studies are important. As the body of evidence on trichloroethylene has grown over the past 20 years, so has the number of studies with clearly articulated hypothesis. All Nordic cohort studies (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001; Raaschou-Nielsen et al., 2003) are designed to examine cancer and TCE, albeit some with limited statistical power, as are recent cohort studies of United States occupationally exposed populations (Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999, 2006a; Zhao et al., 2005; Radican et al, 2008). Exposure assessment approaches in these studies distinguished subjects with varying potentials for TCE exposure, and in some cases, assigned a semiquantitative TCE exposure surrogate to individual study subjects. Three case-control studies nested in cohorts, furthermore, examined TCE exposure and site-specific cancer, albeit a subject's potential and overall prevalence of TCE exposure greatly varied between these studies (Wilcosky et al., 1984; Greenland et al., 1994; Krishnadasan et al., 2007). Typically, studies of all workers at a plant or manufacturing facility (Shindell and Ulrich, 1985; Shannon et al., 1988; Blair et al., 1989; Sinks et al., 1992; Garabrant et al., 1988; Costa et al., 1989; ATSDR, 2004; Chang et al., 2003, 2005; Sung et al., 2007, 2008; Clapp and Hoffman, 2008) are not designed to evaluate cancer and TCE specifically, given their inability to identify varying TCE exposure potential for individual study subjects; rather, such studies evaluate the health status of the entire population working at that facility. Bias associated with exposure misclassification is greater in these studies, and for this and other reasons more fully discussed below, they are of limited utility for informing evaluations on TCE exposure and cancer.

Recent case-control studies with hypotheses specific for TCE exposure include the kidney cancer case-control studies of Vamvakas et al. (1998), Brüning et al. (2003), and Charbotel et al. (2006, 2009). More common, population-based case-control studies assess occupational exposure to organic solvents, using a job-exposure matrix approach for exposure assessment to examine organic solvent categories, i.e., aliphatic hydrocarbons, or specific solvents such as TCE. The case-control studies of Costas et al. (2002; childhood leukemia) and Lee et al. (2003; liver cancer) were also designed to examine possible association with contaminated drinking water containing trichloroethylene and other solvents detected at lower concentrations. The hypothesis of Siemiatycki (1991) and ancillary publications (Siemiatycki et al., 1994; Fritschi and Siemiatycki, 1996a, b; Dumas et al., 2000; Parent et al., 2000a, b; Goldberg et al., 2001) explored possible association between 20 site-specific cancers and occupational title or chemical exposures, including TCE exposure, using a contemporary exposure assessment approach for more focused research investigation.

Cases and control selection in most population-based case-control studies of TCE exposure are considered a random sample and representative of the source population (Siemiatycki, 1991 [and related publications, Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996a, b; Dumas et al., 2000; Parent et al., 2000a, b; Goldberg et al., 2001]; Lowengart et al., 1987; McKinney et al., 1991; Hardell et al., 1994; Heineman et al., 1994; Nordstrom et al., 1998; Dosemeci et al., 1999; Kernan et al., 1999; Persson and Fredriksson, 1999; Pesch et al., 2000a, b; De Roos et al., 2001; Costas et al., 2002; Brüning et al., 2003; Lee et al., 2003; Shu et al., 2004; Charbotel et al., 2006, 2009; Miligi et al., 2006; Seidler et al., 2007; Constantini et al., 2008; Wang et al., 2009]). Case and control selection in Vamvakas et al. (1998), a study conducted in the Arnsberg area of Germany, is subject to criticism regarding possible selection bias resulting from differences in selection criteria, cases worked in small industries and controls from a wider universe of industries; differences in age, controls being younger than cases with possible lower exposure potentials; and temporal difference in case and control selection, controls selected only during the last year of the study period with possible lower exposure potential if exposure has decreased over period of the study (NRC, 2006). The potential for selection bias in Brüning et al. (2003), another study in the same area as Vamvakas et al. (1998) but of later period of observation, was likely reduced compared to Vamvakas et al. (1998) due to the broader region of southern Germany from which cases were identified and interviewing cases and controls during the same time. One case-control study nested in a cohort (Greenland et al., 1994) included subjects whose deaths were reported to and known by the employer, e.g., occurred among vested or pensioned employees or among currently employees. A 10- to 15-year employment period was required for employees in this study to receive a pension; deaths among employees who left employment before this time were not known to the employer and not included the study. Survivor bias, a selection bias, may be introduced by excluding nonpensioned workers or those who leave employment before becoming vested in a company's retirement plan is more likely than in a study of all employees with complete follow-up. The use of pensioned deaths as controls, as was done in this study, would reduce potential bias if both cases and control had the same likelihood of becoming pensioned. That is, the probability for becoming a pensioned worker is similar for all deaths and unrelated to the likelihood of exposure or magnitude of exposure and disease. No information was available in Greenland et al. (1994) to evaluate this assumption.

Geographic-based and ecological studies of TCE contaminated water supplies typically focus on estimating cancer or other disease rates in geographically circumscribed populations who are geospatially located with a source containing TCE, e.g., a hazardous waste site, well water, or air. These studies are often less informative for studying cancer due to their inability to estimate incidence rate ratios, essential for causal inferences, inferior exposure assessment approach, and to possible selection biases. Ecological studies also are subject to bias known as "ecological fallacy" since variables of exposure and outcome measured on an aggregate level do not represent association at the individual level. Consideration of this bias is important for diseases with more than one risk factor, such as the site-specific cancers evaluated in this assessment.

B.2.2. Outcomes Assessed in Trichloroethylene (TCE) Epidemiologic Studies

The epidemiologic studies consider at least three levels of health outcomes in their examinations of human health risks associated with exposure to trichloroethylene: biomarkers of effects and susceptibility, morbidity, and mortality (NRC, 2006). Few susceptibility biomarkers have been examined and these are not specific to trichloroethylene (NRC, 2006). By far, the bulk of the literature on cancer and trichloroethylene exposure is of cancer morbidity (Isacson et al., 1985; Lowengart et al., 1987; Shannon et al., 1988; Fredriksson et al., 1989; AZ DHS, 1990, 1995; McKinney et al., 1991; Siemiatycki, 1991; Persson et al., 1993; Persson and Fredriksson, 1999; Vartiainen et al., 1993; Axelson et al., 1994; Cohn et al., 1994; Hardell et al., 1994; Anttila et al., 1995; Nordstrom et al., 1998; Vamvakas et al., 1998; Dosemeci et al., 1999; Dumas et al., 2000; Pesch et al., 2000a, b; De Roos et al., 2001; Hansen et al., 2001; Costas et al., 2002; Morgan and Cassady, 2002; Brüning et al., 2003; Rasschou-Nielsen et al., 2003; Aickin, 2004; Shu et al., 2004; Coyle et al., 2005; ATSDR, 2006a; Charbotel et al., 2006, 2009; Miligi et al., 2006; Seidler et al., 2007; Sung et al., 2008; Wang et al., 2009), mortality (Wilcosky et al., 1984; Shindell and Ulrich, 1985; Garabrant et al., 1988; Blair et al., 1989; Costa et al., 1989; Kernan et al., 1999; Aickin et al., 1992; Greenland et al., 1994; Heineman et al., 1994; Morgan et al., 1998; Boice et al., 1999, 2006a; Ritz, 1999a; Lee et al., 2003; ATSDR, 2004;; Clapp and Hoffman, 2008, Radican et al, 2008) or both (Sinks et al., 1992; Henschler et al., 1995; Blair et al., 1998; Chang et al., 2003, 2005; Sung et al., 2007; Zhao et al., 2005).

Mortality is readily identified from death certificates; however, diagnostic accuracy from death certificates varies by the specific diagnosis (Brenner and Gefeller, 1993). Incident cancer cases are enumerated more accurately by tumor registries and by hospital pathology records and cases identified from these sources are considered to have less bias resulting from disease misclassification than cause or underlying cause of death as noted on death certificates. Studies of incidence are preferred, particularly for examining association with site-specific cancers having high 5-year survival rates or which may be misclassified on death certificate. Misclassification of the cause of death as noted on death certificates attenuates statistical power through errors of outcome identification. This nondifferential misclassification of outcome in cohort studies will lead to attenuation of rate ratios, although the magnitude of is difficult to predict (NRC, 2006). Cancer registries are used for cases diagnosed in more recent time periods and cohorts whose entrance dates are 30 or 40 years may miss many incident cancers and reduced statistical power as a consequence. Two studies examine both cancer incidence and mortality (Blair et al., 1998; Zhao et al., 2005). The lapse of 20 or more years in Blair et al. (1998) and 38 years in Zhao et al. (2005) between date of cohort identification and cancer incidence ascertainment suggests these studies are missing cases and limits incidence examinations.

B.2.3. Disease Classifications Adopted in Trichloroethylene (TCE) Epidemiologic Studies

Disease coding and changes over time are important in epidemiologic evaluations, particularly in evaluation of heterogeneity or consistency of observations from a body of evidence. The ICD, published by WHO, is used to code underlying and contributing cause of death on death certificates and is updated periodically, adding to diagnostic inconsistency for cross-study comparisons (NRC, 2006). Tumor registries use the International Classification of Diseases-Oncology (ICD-O) for coding the site and the histology of neoplasms, principally obtained from a pathology report.

The epidemiologic studies of TCE exposure have used a number of different classification systems (Scott and Chiu, 2006). A number of studies classified neoplasms according to ICD-O (Siemiatycki, 1991; Costas et al., 2002) or to ICD-9 (Nordstrom et al., 1998; Kernan et al., 1999; Ritz, 1999a; Chang et al., 2005; Zhao et al., 2005). Other ICD revisions used in recent studies include ICDA-8 (Blair et al., 1989; Greenland et al., 1994; Blair et al., 1998), ICD-7 (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001; Raaschou-Nielsen et al., 2003), or several ICD revisions, whichever was in effect at the date of death (Garabrant et al., 1988; Morgan et al., 1998; Boice et al., 1999, 2006a; Radican et al., 2008). In this latter case, changes in disease classification over revisions are not harmonized or recoded to a common classification; and, diagnostic inconsistencies and disease misclassification errors leads to a greater likelihood for bias in these studies. Greatest weight is placed on studies where all cases or deaths are classified using current classification systems. However, association in studies adopting older revisions, ICD 7 (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001; Raaschou-Nielsen et al., 2003), for example, is noteworthy given the narrow consideration of lymphoid neoplasms compared to contemporary classification systems. Consistency examinations of the overall body of evidence using meta-analysis methods and examination of heterogeneity will need to consider study differences in coding in interpreting findings.

A major shift in thinking occurred around 1995 with the Revised European-American Lymphoma (REAL) classification of grouping diseases of the blood and lymphatic tissues along their cell lines compared to previous approaches to group lymphomas by a cell's physical characteristics. It was increasing recognized that some lymphomas and corresponding lymphoid leukemias were different phases (solid and circulating) of the same disease entity (Morton et al., 2007). Many concepts of contemporary knowledge of lymphomas are incorporated in the WHO Classification of Neoplastic Diseases of the Hematopoietic and Lymphoid Tissues, an international consensus scheme for classifying leukemia and lymphoma now in use and the predecessor to REAL (Jaffe et al., 2001). Both the ICD-O, 3rd edition, and ICD-10 have adopted the WHO classification framework.

The only study coding lymphomas using the WHO classification is Seidler et al. (2007). Other lymphoma studies have adopted older lymphoma classification systems, either the National Cancer Institute's (NCI) Working Formulation (Miligi et al., 2006; Costantini et al., 2008) or other systems coding lymphomas according to NCI's Working Formulation, i.e., International Classification of Disease-Oncology, 2nd Edition (Wang et al., 2009), that divided lymphomas into low-grade, intermediate-grade and high grade, with subgroups based on cell type and presentation, or Rappaport (Hardell et al., 1994, 1981), with groupings based on microscopic morphology (Lymphoma Information Network, 2008). Lowengart et al. (1987), Persson et al. (1989, 1993), McKinney et al. (1991) nor Persson and Fredriksson (1999) provide information in their published articles on lymphomas classification systems used in these studies.

Implications of classification changes are most significant for lymphoma. As noted by the IOM (2003), in Revision 7 and earlier editions of the ICD, all lymphatic and hematopoietic neoplasms were grouped together instead of treated as individual types of cancer (such as Hodgkin's disease) or specific cell types (such as acute lymphocytic leukemia). One limitation of this treatment was the amalgamation of these relatively rare cancers would increase the apparent sample size but could also result in diluted estimates of effect if etiologic heterogeneity of different lymphoma subtypes existed, i.e., different sites of cancer were not associated in similar ways with the exposures of interest. Additionally, immunophenotyping was not available, leading to decreased ability to distinguish ambiguous diseases, and diagnoses of these cancers may have been misclassified; for example, NHL may have been grouped with other lymphatic and hematopoietic cancers to increase statistical power or misclassified as Hodgkin's disease, for example. Examination of distinct lymphoma subtypes is expected to reduce disease misclassification bias. Two case-control studies on non-Hodgkin's lymphoma (NHL) include analysis of lymphoma subtype and trichloroethylene exposure (Miligi et al., 2006; Seidler et al., 2007).

A change in liver cancer coding occurred between ICDA-8 and ICD-9 and is important to consider in examinations of liver cancer observations across the TCE studies. With ICD-9, liver cancer "not specified as primary or secondary" was moved from the grouping of secondary malignant neoplasms and added to the larger class of malignant liver neoplasms. Thus, a similar grouping of liver cancer causes is necessary to cross-study comparisons. For example, an examination of liver cancer, based on ICDA-8, would need to include codes for liver and intrahepatic bile duct (code 155) and liver, not specified as primary or secondary (code 197.8), but, for ICD-9, would include liver and intrahepatic bile duct (code 155) only. The effect of adding "liver cancer, not specified as primary or secondary" to the larger liver and intrahepatic bile duct category in ICD-9 was a 2-fold increase in the overall liver cancer mortality rate (Percy et al., 1990).

B.2.4. Exposure Classification

Adequacy of exposure assessment approaches and their supporting data are a critical determinant of a study's contribution in a weight-of-evidence evaluation (Checkoway et al., 1989). Exposure assessment approaches in studies of TCE and cancer vary greatly. At one extreme, studies assume subjects are exposed by residence in a defined geographic area (Isacson et al., 1985; AZ DHS, 1990, 1995; Aickin et al., 1992, Aickin, 2004; Vartiainen et al., 1993; Cohn et al., 1994; Morgan and Cassidy, 2002; Lee et al., 2003; Coyle et al., 2005; ATSDR, 2006a, 2008) or by employment in a plant or job title (Shindell and Ulrich, 1985; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Chang et al., 2003, 2005; ATSDR, 2004; Sung et al., 2007, 2008; Clapp and Hoffman, 2008). This is a poor exposure surrogate given potential for TCE exposure can vary in these broad categories depending on job function, year, use of personal protection, and, for residential exposure, pollutant fate and transport, water system distribution characteristics, percent of time per day in residence, presence of mitigation devices, drinking water consumption rates, and showering times. Another example comprises measurement from a subset of workers with jobs where TCE is routinely used to infer TCE exposure and TCE intensity to all subjects. In both examples, exposure misclassification potential may be extensive and with a downward bias in risk estimates.

At the other extreme and preferred given a reduced likelihood for misclassification bias, quantitative exposure assessment based upon a subject's job history, job title, and monitoring data are used to develop estimates of TCE intensity and cumulative exposure (quantitative exposure metrics or measures) and is know as job-exposure matrix (JEM) approaches. Peak exposure is also well characterized. Addition to JEM approaches of information on job tasks (JTEM) associated with exposure such as that done by Pesch et al. (2000a, b) is expected to reduce potential exposure misclassification. In between these two extremes, semiquantitative estimates of low, medium, and high TCE exposure are assigned to subjects. Twelve studies assigned a quantitative or semiquantitive TCE surrogate metrics to individual subjects using a JEM or job-task-exposure-matrix (JTEM): Siemiatycki (1991 [and related publications, Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996a, b; Dumas et al., 2000; Parent et al., 2000a, b; Goldberg et al., 2001]), Blair et al. (1998) and follow-up by Radican et al. (2008), Morgan et al. (1998), Vamvakas et al. (1998), Kernan et al. (1999), Ritz (1999a), Pesch et al. (2000a, b), Brüning et al. (2003), Zhao et al. (2005), Charbotel et al. (2006, 2009), Krishnadansen et al. (2007), Seidler et al. (2007), and Wang et al. (2009).

Fifteen other studies assigned a qualitative TCE surrogate metric (ever exposed or never exposed), less preferred to a semi-quantitative exposure surrogate given greater likelihood for error associated exposure misclassification, using general job classification of job title by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, job exposure matrices, water distribution models, for cohort studies, or obtained from subjects using questionnaire for case-control studies. The 15 studies were: Wilcosky et al. (1984), Lowengart et al. (1987), McKinney et al. (1991), Greenland et al. (1994), Hardell et al. (1994), Nordstrom et al. (1998), Shu et al. (1999, 2004), Boice et al. (1999, 2006a), Dosemeci et al. (1999), Persson and Fredriksson (1999), Costas et al. (2002), Raaschou-Nielsen et al. (2003), Miligi et al. (2006), and Costantini et al. (2008). Without quantitative measures, however, it is not possible to quantify exposure difference between groupings nor is it possible to compare similarly named categories across studies. Exposure misclassification for dichotomous exposure defined in these studies, if nondifferential, would downward bias resulting risk estimates.

Zhao et al. (2005), Krishnadansen et al. (2007), and Boice et al. (2006a) are studies with overlap in some subjects, but with different exposure assessment approaches, more fully discussed in B.3.1.1., with implication on study ability to identify cancer hazard. While these studies used job title to assign TCE exposure potential, Zhao et al. (2005) and Krishnadansen et al. (2007) developed a semiquantitative estimate of TCE exposure potential, whereas, Boice et al. (2006a) classified subjects as either “exposed” or “unexposed” using a qualitative surrogate. These studies, furthermore, identify TCE exposure potentially differently for possibly similar job titles. For example, jobs as instrument mechanics, inspectors, test stand engineers, and research engineers are identified with medium potential exposure in Zhao et al. (2005) and Krishnadansen et al. (2007); however, these job titles were considered in Boice et al. (2006a) as having background exposure and were combined with unexposed subjects, the referent population in Cox Proportional Hazard analyses.

Three Nordic cohorts have TCE exposure as indicated from biological markers, assigning TCE exposure to subjects using either concentration of trichloroacetic acid (TCA) in urine or TCE in blood (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001). The utility of a biomarker depends on it selectivity and the exposure situation. Urinary TCA (U-TCA) is a nonselective marker since other chlorinated solvents besides TCE are metabolized to TCA and resultant urinary elimination. If only TCE is the only exposure, urinary TCE may be a useful marker; however, in setting with mixed exposure, urinary TCA may serve as an integrated exposure marker of several chlorinated solvents. The Nordic studies used the linear relationship found for average inhaled trichloroethylene versus U-TCA: trichloroethylene (mg/m^3) = 1.96; U-TCA (mg/L) = 0.7 for exposures lower than 375 mg/m^3 (69.8 ppm) (Ikeda et al., 1972). This relationship shows considerable variability among individuals, which reflects variation in urinary output and activity of metabolic enzymes. Therefore, the estimated inhalation exposures are only approximate for individuals but can provide reasonable estimates of group exposures. There is evidence of nonlinear formation of U-TCA above about 400 mg/m^3 or 75 ppm of trichloroethylene. The half-life of U-TCA is about 100 hours. Therefore, the U-TCA value represents roughly the weekly average of exposure from all sources, including skin absorption. The Ikeda et al. (1972) relationship can be used to convert urinary values into approximate airborne concentration, which can lead to misclassification if tetrachloroethylene and 1,1,1-trichloroethane are also being used because they also produce U-TCA. In most cases, the Ikeda et al. (1972) relationship provides a rough upper boundary of exposure to trichloroethylene.

B.2.5. Follow-up in Trichloroethylene (TCE) Cohort Studies

Cohort studies are most informative if vital status is ascertained for all cohort subjects and if the period of time for disease ascertainment is sufficient to allow for long latencies, particularly for cancer detection and death, in the case of mortality studies. Inability to ascertain vital status for all subjects, or, conversely, subjects who are loss-to-follow-up, can affect the validity of observations and lead to biased results. Both power and rate ratios estimated in cohort studies can be underestimated due to bias introduced if the follow-up period was not long enough to account for latency (NRC, 2006). The probability of loss to follow-up may be related to exposure, disease, or both. The multiple-stage process of cancer development occurs over decades after first exposure and studies with full latent periods are considered to provide greater weight to the evaluation compared to cohort studies with shortened follow-up period and lower percentage of subjects whose vital status was known on the date follow-up ended. Vital status ascertainment for over 90% of all cohort studies and long mean follow-up periods, say 15 years of longer, characterized many occupational cohort studies on trichloroethylene and cancer (Garabrant et al., 1988; Costa et al., 1989; Anttila et al., 1995; Blair et al., 1998 and the follow-up study of Radican et al., 2008; Morgan et al., 1998; Boice et al., 1999, 2006a; Ritz, 1999a; Raaschou-Nielsen et al., 2003; Zhao et al., 2005). Information is lacking in two biomarker studies (Axelson et al., 1994; Hansen et al., 2001), additionally, to estimate the mean follow-up period for TCE-exposed subjects; although, Hansen et al. (2001) state "some workers were followed for as long as 50 years after their exposure, which allowed the detection of cancers with long latency periods." Other studies of trichloroethylene and cancer did not identify a latent period, information for calculating a latent period, or contained other deficiencies in follow-up criteria (Wilcosky et al., 1984; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Sinks et al., 1992; Henschler et al., 1995; Chang et al., 2005; Sung et al., 2007). Proportionate mortality ratio studies, based only on deaths and which lack information on person-year structure as cohort studies, by definition, do not contain information on cancer latent periods or follow-up (ATSDR, 2004; Clapp and Hoffman, 2008).

B.2.6. Interview Approaches in Case-Control Studies of Cancer and Trichloroethylene (TCE) Exposure

Interview approaches and the percentage of subjects with information obtained from proxy or next-of-kin respondents need consideration in interpreting population and hospital-based case-control studies in light of possible biases. Biases resulting from proxy respondent or from low participation related to mailed questionnaires are not relevant to cohort or geographic studies since information is obtained from local, national, or corporate records. Both face-to-face and telephone interviews are common and valid approaches used in population or hospital-based case-control studies. Important to each is the use of a structured questionnaires combined with intensive training as ways to minimize a high potential for biases often associated with mailed questionnaires (Schlesselman, 1982; Blatter et al., 1997). Studies with information limited to job title, type of business and dates of employment and aided with computer or job-exposure-matrix approaches are preferred to studies of job title only; the added approaches can reduce exposure misclassification bias and improve disease risk estimates (Stewart et al., 1996). Moreover, interview with respondents other than the individual case or control, through proxy or next-of-kin respondents, may also introduce bias in case-control studies. Proxy respondents are used when cases or control are either too sick to respond or if deceased. This bias would dampen observed associations if proxy respondents did not fully provide accurate information. Boyle et al. (1992), for example, in their study of several site-specific cancers and occupational exposures found low sensitivity, or correct reporting, for occupational exposure to solvents among proxy respondents. The weight of evidence analysis on trichloroethylene and cancer, for this reason, places greatest weight on observations from studies which obtain information on personal, medical, and occupational histories from each case and control with lesser weight is placed on studies where 10 percent or more of interviews are with proxy respondents.

Many of the more recent case-control studies include face-to-face (McKinney et al., 1991; Siemiatycki, 1991; Vamvakas et al., 1998; Dosemeci et al., 1999; Costas et al., 2002; Pesch et al., 2000a, b; Brüning et al., 2003; Miligi et al., 2006; Seidler et al., 2007; Wang et al., 2009) or telephone (Lowengart et al., 1987; Shu et al., 1999, 2004; Charbotel et al., 2006, 2009) interviews. Few of these studies included interviewers who were blinded or did not know the identity of who is a case and who is a control; although, many studies assigned exposure to cases and controls in a blinded manner. Information obtained from mailed questionnaire predominantly characterized older Nordic studies (Hardell et al., 1981, 1994; Fredriksson et al., 1989; Persson et al., 1989, 1993; Persson and Fredriksson, 1999; Nordstrom et al., 1998). One case-control study did not ascertain information from a questionnaire or through interviews, instead using occupation coded on death certificates to infer TCE exposure potential (Kernan et al., 1999). In all studies except Costas et al. (2002) and Kernan et al. (1999), assignment of potential TCE exposure to cases and controls, to different degrees depending on each study, is based on self-reported information on job title, and in some cases, to specific chemicals.

More common to the case-control studies on trichloroethylene and cancer was possible bias related to a higher percentage of proxy interviews. Four studies (Dosemeci et al., 1999; Pesch et al., 2000a, b; Wang et al., 2009) excluded subjects with proxy interviews and the percentage of proxy interview among subjects in one other study is less than 10 percent (Nordstrom et al., 1998). Charbotel et al. (2006, 2009) furthermore presents analyses for data they considered as better quality, including higher confidence exposure information and excluding proxy respondents, in addition to analyses using both living and proxy respondents. A consideration of proxy interviews in studies of childhood cancers which include an examination of paternal occupational exposure is needed given a greater likelihood for bias if fathers are not directly interviewed and the father's occupational information is provided only by the child's mother. A good practice is for statistical analyses examining paternal occupational exposure to included only cases and controls with direct information provided by the fathers, such as De Roos et al. (2001), the only childhood cancer study (neuroblastoma) to exclude the use of proxy information.

B.2.7. Sample Size and Approximate Statistical Power

Cancer is generally considered a rare disease compared to more common health outcomes such as cardiovascular disease. Of all site-specific cancers, endocrine cancers of the breast prostate and lung cancer are most common, with age-adjusted incidence rates of 126.0 per 100,000 women (breast), 163 per 100,000 men (prostate), and 63.9 per 100,000 men and women (lung) (Ries et al., 2008). Several site-specific cancers including kidney cancer, liver cancer, and lymphoma that are of interest to trichloroethylene are rarer and consideration of study size and the influence on statistical power are factors for judging a study's validity and assessment of a study's contribution to the overall weight-of-evidence for identifying a hazard. For example, the age-adjusted incidence rates of non-Hodgkin's lymphoma, liver and intrahepatic bile duct cancer, and kidney and renal pelvis cancer in the United States population are 19.5 per 100,000, 6.4 per 100,000, and 13.2 per 100,000; rates vary by sex and race. Age-adjusted mortality rates for these cancers are lower: 7.3 per 100,000 (NHL), 5.0 per 100,000 (liver and intrahepatic bile duct), 4.2 per 100,000 (kidney and renal pelvis). Rates of the childhood cancer, acute lymphocytic leukemia, are even lower: 1.6 (incidence) and 0.5 (mortality) per 100,000 (Ries et al., 2008).

Only very large cohort or case-control studies would have a sufficient number of cases and statistical power to estimate excess risks and exposure-response relationships (NRC, 2006). Observations from studies with large numbers of TCE-exposed subjects, given consideration of exposure conditions and other criteria discussed in this section, can provide useful information on hazard and may provide quantitative information on possible upper bound trichloroethylene cancer risks. Alternatively, studies of small numbers of subjects or cases and controls, typically, studies with statistical power less than 80% to detect risk of a magnitude of 2 or less, are not likely to provide useful evidence for or against the hypothesis that trichloroethylene is a human carcinogen.

Studies with either a large number of TCE-exposed subjects or with large numbers of total deaths, cancer deaths, or cancer cases among TCE-exposed subjects are the cohort studies of Blair et al. (1998), Raaschou-Nielsen et al. (2003), and Zhao et al. (2005), and the case-control studies of Pesch et al. (2000a), Shu et al. (1999, 2004 [paternal exposure assessment, only]), Miligi et al. (2006), and Seidler et al. (2007). The cohorts of Boice et al. (1999, 2006a) and Morgan et al. (1998), like that of Blair et al. (1998), comprised over 10,000 subjects both with and without potential TCE exposure; however, the number of subjects and the percentage of the larger cohort identified with TCE exposure in these studies was less than that in Blair et al. (1998); 23% of all subjects in Morgan et al. (1998), 3% in Boice et al. (1999), 2% in Boice et al. (2006a) compared to 50% in Blair et al. (1998). Moreover, although the cohorts of Garabrant et al. (1988), Chang et al. (2005) and Sung et al. (2007) are also of population sizes greater than 10,000, these studies of employees at one manufacturing facility lack assignment of potential TCE exposure to individual subjects and include subjects with varying exposure potential, some of whom are likely with very low to no exposure potential to TCE. Rate ratios estimated from cohorts that include unexposed subjects would be underestimated due; although the magnitude of this bias can not be calculated given the absence in individual studies of information on the percentage of subjects lacking potential TCE exposure.

Examination of the statistical power or ability to detect a rate ratio magnitude for site-specific cancer in an epidemiologic study informs weight-of-evidence evaluations and provides perspective on a study's validity and robustness of observations. Although statistical power calculations are traditionally carried out during the design phase for sample size estimation, examination of a study's statistical power *post hoc* is one of several tools to evaluate a study's validity; however, such calculations must be interpreted in context of exposure conditions in the study. Given the lower average exposure concentrations in the cohort studies and in population case-control studies, an assumption of low relative risks is plausible. Approximate statistical power to detect a relative risk of 2.0 with $\alpha = 0.05$ was calculated for site-specific cancers in cohort and geographic-based studies according to the methods of Beaumont and Breslow (1981), as suggested by NRC (2006), and are found in Table B-4. Approximate statistical power was calculated for kidney, NHL, and liver cancers as examples. Radican et al. (2008), the previously follow-up of this cohort by Blair et al. (1998), and Raaschou-Nielsen et al. (2003) have over 80% statistical power to detect relative risk of 2.0 for kidney and liver cancers and NHL and overall TCE exposure. However, while these studies may appear sufficient for examining overall TCE exposure and relative risks of 2.0, they have a greatly reduced ability to detect underlying risks of this magnitude in analyses using rank-ordered exposure- or duration-response analyses. Other studies with fewer TCE-exposed subjects and of similar or lower exposure conditions as Blair et al. (1998) will decreased statistical power to detect most site-specific cancer risks of less than 2.0. Statistical power in Morgan et al. (1998, 2000) and Boice et al. (1999) approaches that in Blair et al. (1999) and Raaschou-Nielsen et al. (2003). As further identified in Table B-4, Garabrant et al. (1988) and Morgan and Cassady each had over 80% statistical power to detect relative risks of 2.0 for liver and kidney cancer and reflects the number of subjects in each of these studies. However, underlying risk in both studies and other studies such as these which lack characterization of TCE exposure to individual subjects is likely lower than 2.0 because of inclusion of subjects with varying exposure potential, including low exposure potential. Case-control studies such as Charbotel et al. (2006) and Brüning et al. (2003) examine higher level exposure to TCE than average exposure in the population case-control studies, and although these two studies contain fewer subjects than population case-control studies such as Seidler et al. (2007), a higher statistical power is expected related to the different and higher exposure conditions and to the higher prevalence of exposure.

Overall, except for a few studies noted above, the body of evidence has limited statistical power for evaluating low level cancer risk and trichloroethylene. For this reason, studies reporting statistically significant association between trichloroethylene and site-specific cancer are noteworthy if positive biases such as confounding are minimal.

B.2.8. Statistical Analysis and Result Documentation

Appropriate analysis approaches characterize most cohort and case-control studies on trichloroethylene cancer. Many studies clearly documented statistical analyses, evaluated possible confounding factors, and included an examination of exposure-response. In occupational cohort studies, potential confounding factors other than age, sex, race, and calendar year are, generally, not evaluated. Expected numbers of outcomes (deaths or incident cancers) were calculated using life table analysis and an external comparison group, national or regional population mortality or incidence rates (Shindell and Ulrich, 1985; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Sinks et al., 1992; Axelson et al., 1994; Anttila et al., 1995; Henschler et al., 1995; Morgan et al., 1998; Blair et al., 1998; Boice et al., 1999, 2006a; Raaschou-Nielsen et al., 2003; Chang et al., 2003, 2005; ATSDR, 2004; Sung et al., 2007). Risk ratios are also presented in some cohort studies using proportional hazard and logistic regression statistical methods using mortality or incidence rates of non-TCE exposed cohort subjects as referent or internal controls (Ritz, 1999a; Blair et al., 1998; Boice et al., 1999, 2006a; Zhao et al., 2005, Radican et al., 2008). Use of a non-TCE exposed referent group employed at the same facility as exposed generally reduces downward bias or bias having potential associations masked by a healthy worker work or other factors that may be more similar within an occupational cohort than between the cohort and the general population. However, the advantage is minimized if subjects with lower TCE exposure potential are included in the referent group as in Boice et al. (2006a). One referent group (the SSFL group) of Boice et al. (2006a) included individuals with low TCE potential, a treatment different from the overlapping study of Zhao et al. (2005) whose exposure assessment adopted a semi-quantitative approach, grouping subjects identified with low TCE exposure potential separately from subjects with no TCE exposure potential. A second referent group of all Rocketdyne workers in Boice et al. (2006a) for whom TCE exposure potential was not examined may, also, have potential for greater than background exposure since TCE use was widespread and rocket engine cleaning occurred at other locations besides at test sites (Morgenstern et al., 1999).

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2

Exposure group	NHL	Kidney	Liver	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al., 2005
Any exposure to TCE	Not reported	Not reported	Not reported	
Low cumulative TCE score	Referent	Referent	Referent	
Medium cumulative TCE score	97.0	43.8	Not reported	
High TCE score	58.2	18.7	Not reported	
All employees at electronics factory (Taiwan)				Chang et al., 2005
Males	Not reported	Not reported	16.9	
Females	Not reported	92.1[a]	15.4	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
Any exposure, all subjects	100.0	100.0	100.0	
Employment duration, males				
<1 yr	98.4	96.6	85.2	
1−4.9 yrs	99.4	98.4	92.7	
≥5 yrs	97.7	97.0	93.1	
Employment duration, females				
<1 yr	40.3	30.1	27.3	
1−4.9 yrs	48.4	37.1	34.1	
≥5 yrs	39.6	31.9	30.5	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group	NHL	Kidney	Liver	Reference
Biologically-monitored Danish workers				Hansen et al., 2001
Any TCE exposure	37.9	47.9	35.7	
Cumulative exposure (Ikeda)		Not reported	Not reported	
<17 ppm-yr	17.9			
≥17 ppm-yr	20.3			
Mean concentration (Ikeda)		Not reported	Not reported	
<4 ppm	21.0			
4+ ppm	23.6			
Employment duration		Not reported	Not reported	
<6.25 yr	18.3			
≥6.25	20.1			
Aircraft maintenance workers from Hill Air Force Base				Blair et al., 1998
TCE subcohort	Not reported	Not reported	Not reported	
Males, cumulative exposure				
0	Referent	Referent	Referent	
<5 ppm-yr	79.5	67.8	58.2	
5−25 ppm-yr	63.1	49.4	44.7	
>25 ppm-yr	70.8	58.4	47.4	
Females, cumulative exposure				
0	Referent	Referent	Referent	
<5 ppm-yr	28.2	0 cases	0 cases	
5−25 ppm-yr	0 cases	0 cases	0 cases	
>25 ppm-yr	34.1		0 cases	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group		NHL	Kidney	Liver	Reference
Biologically-monitored Finnish workers					Anttila et al., 1995
	All subjects	53.8	70.4	56.5	
	Mean air-TCE (Ikeda extrapolation)				
	<6 ppm	36.8	Not reported	23.2	
	6+ ppm	25.6	Not reported	17.4	
Cardboard manufacturing workers in Arnsberg, Germany					Henschler et al., 1995
	Exposed workers	Not reported	16.3	Not reported	
Biologically-monitored Swedish workers					Axelson et al., 1994
	Any TCE exposure, males	43.5	59.6	0.05	
	Any TCE exposure, females	Not reported	Not reported	Not reported	
Cardboard manufacturing workers, Atlanta area, GA					Sinks et al., 1992
	All subjects	Not reported	27.9	Not reported	
Cohort studies—mortality					
Aerospace workers (Rocketdyne)					
	Any TCE (utility/engine flush)	56.0	43.5	42.6	Boice et al., 2006a
	Any exposure to TCE	Not reported	Not reported	Not reported	Zhao et al., 2005
	Low cumulative TCE score	Referent	Referent	Referent	
	Medium cumulative TCE score	97.0	57.6	Not reported	
	High TCE score	55.4	26.4	Not reported	
View-Master employees					ATSDR, 2004
	Males	40.9	17.3	23.4	
	Females	74.1	24.1	0 deaths	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group	NHL	Kidney	Liver	Reference
All employees at electronics factory (Taiwan)				Chang et al., 2003
Males	49.8	0 deaths	16.9	
Females	79.0	37.5	15.4	
United States uranium-processing workers (Fernald)				Ritz, 1999a
Any TCE exposure				
Light TCE exposure, >2 yrs duration	91.6[b]	59.7[c]	10.1	
Mod. TCE exposure, >2 yrs duration	20.9[b]	0 deaths[c]	0.08	
Aerospace workers (Lockheed)				Boice et al., 1999
Routine exposure	88.4	71.3	72.9	
Duration of exposure, routine-intermittent				
0 yrs	Referent	Referent	Referent	
<1 yr	81.7	66.3	73.6	
1−4 yrs	73.5	60.3	63.5	
≥5 yrs	78.5	63.8	67.3	
p for trend				
Aerospace workers (Hughes)				Morgan et al., 1998
TCE subcohort	42.6, 79.6[d]	65.5	65.6	
Low intensity (<50 ppm)	22.1	33.3	34.7	
High intensity (>50 ppm)	31.8	50.1	49.2	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group	NHL	Kidney	Liver	Reference
Aircraft maintenance workers (Hill AFB, UT)				Blair et al., 1998
TCE subcohort	92.7	81.5	87.9	
Males, cumulative exposure				
0				
<5 ppm-yr	62.1	50.7	61.4	
5−25 ppm-yr	43.1	37.1	44.7	
>25 ppm-yr	54.8	44.9	52.8	
Females, cumulative exposure				
0				
<5 ppm-yr	18.2	0 deaths	0 deaths	
5−25 ppm-yr	0 deaths	8.4	0 deaths	
>25 ppm-yr	22.0	11.5	19.1	
TCE subcohort	99.9	94.4	99.7	Radican et al., 2008
Males, cumulative exposure				
0				
<5 ppm-yr	83.0	43.8	59.4	
5−25 ppm-yr	64.9	53.0	70.6	
>25 ppm-yr	75.7	33.4	50.9	
Females, cumulative exposure				
0				
<5 ppm-yr	38.9	0 deaths	25.9	
5−25 ppm-yr	0 deaths	12.4	0 deaths	
>25 ppm-yr	49.2	21.1	32.2	
Cardboard manufacturing workers in Arnsberg, Germany				Henschler et al., 1995
TCE exposed workers	19.6[b]	16.0	Not reported	

Table B-4. Approximate statistical power (%) in cohort and geographic-based studies to detect an RR = 2 (continued)

Exposure group	NHL	Kidney	Liver	Reference
Cardboard manufacturing workers, Atlanta area, GA	45.3[b]	17.3	Not reported	Sinks et al., 1992
Coast Guard employees (US)				Blair et al., 1989
Marine inspectors	31.8	31.8	38.6	
Aircraft manufacturing plant employees (Italy)				Costa et al., 1989
All subjects	94.1[b]	Not reported	63.1	
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
All subjects	95.1[e], 74.2[f]	90.9	77.9	
Geographic based studies				
Residents in two study areas in Endicott, NY	90.8	41.7	31.8	ATSDR, 2006
Residents of 13 census tracts in Redlands, CA	100	100.0	98.7	Morgan and Cassady, 2002
Finnish residents				Vartiainen et al., 1993
Residents of Hausjarvi	98.8	Not reported	84.2	
Residents of Huttula	98.7	Not reported	83.2	

[a]Kidney cancer and other urinary organs, excluding bladder, as reported in Sung et al. (2008).
[b]All cancers of hematopoietic and lymphatic tissues.
[c]Bladder and kidney cancer, as reported in NRC (2006).
[d]Based on number of observed cases of NHL reported in Mandel et al. (2006).
[e]Lymphosarcoma and reticulosarcoma.
[f]Other lymphatic and hematopoietic tissue neoplasms.

Cohort studies additionally evaluate a limited number of other factors associated with employment which could be easily obtained from company and other records such as hire date, time since first employment, socioeconomic status or pay status, and termination date (Greenland et al., 1994; Boice et al., 1999, 2006a; Zhao et al., 2005), and three studies (Ritz, 1999a; Zhao et al., 2005; Boice et al., 2006a) included a limited evaluation of smoking using information collected by survey on smoking patterns from a subgroup of subjects. Neither Morgan et al. (1998) nor Zhao et al. (2005) control for race in analyses, although Morgan et al. (1998) stated that "data concerning race were too sparse to use." The direction of any bias introduced depends on proportion of nonwhites in the referent (internal) group compared to TCE-exposed and on differences between racial groups in site-specific cancer incidence and mortality rates. Blair et al. (1998), furthermore, presumed all subjects of unknown race were white, an assumption with little associated error as shown later by Radican et al. (2008) whose relative risk estimates were adjusted for race in follow-up analysis of this cohort.

The case-control studies on trichloroethylene are better able than cohort studies to evaluate other possible confounders besides age and sex using logistic regression approaches since such information can be obtained directly through interview and questionnaires. The case-control studies of Hardell et al. (1994), Nordstrom et al. (1998) and Persson and Fredriksson (1999) lack evaluation of possible confounding factors other than age, sex and other demographic information used to match control subjects to case subjects. Renal cell carcinoma (RCC) case-control studies included evaluation of suggested risk factors for RCC such as smoking (Siemiatycki, 1991; Vamvakas et al., 1998; Pesch et al., 2000a; Brüning et al., 2003; Charbotel et al., 2006), weight, or obesity (Dosemeci et al., 1999; Charbotel et al., 2006), and diuretics (Vamvakas et al., 1998; Dosemeci et al., 1999). NHL and childhood leukemia case-control studies included evaluation and control for possible confounding due to smoking (Siemiatycki, 1991; Costas et al., 2002; Seidler et al., 2007), alcohol consumption (Costas et al., 2002; Seidler et al., 2007), education (Miligi et al., 2006; Costantini et al., 2008), although etiological factors for these cancers are not well identified other than a suggestion of a role of immune function and some infectious agents in NHL (Alexander et al., 2007).

Mineral oils such as cutting fluids or hydrazine common to some job titles with potential TCE exposure as machinists, metal workers, and test stand mechanics are included as covariates in statistical analyses of Zhao et al. (2005), Boice et al. (2006a) and Charbotel et al. (2006, 2009). In all cases, exposure to cutting oils or to hydrazine did not greatly affect magnitude of risk estimates for TCE exposure.

Geographical studies do not examine possible confounding factors other than sex, age and calendar year. These studies are generally health surveys using publically-available records such as death certificates and lack information on other risk factors such as smoking and exposure to viruses, important to Lee et al. (2003), introduces uncertainties for informing evaluations of trichloroethylene and cancer.

B.2.9. Systematic Review for Identifying Cancer Hazards and Trichloroethylene (TCE) Exposure

The epidemiological studies on cancer and trichloroethylene are reviewed systematically and transparently using criteria to identify studies for meta-analysis. Section B.3 contains a description of and comment on 75 studies of varying qualities for identifying cancer hazard, a question complementary but separate from that examined using meta-analysis. This section identifies of the studies reviewed, studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on job-exposure matrices, biomarker monitoring, or industrial hygiene data indicating a high probability of TCE use) and were judged to have met the inclusion criteria identified below. Lack of inclusion of an individual study in the meta-analysis does not necessarily imply an inability to identify cancer hazard. Not all questions associated with identifying a cancer hazard are addressed using meta-analyses and the 75 studies with varying abilities approached, to sufficient degrees, the standards of epidemiologic design and analysis, identified in the beginning of Section B.2.

The NRC (2006) suggested U.S. EPA conduct a new meta-analysis of the epidemiologic data on trichloroethylene to synthesize the epidemiologic data on TCE exposure. Meta-analysis approaches are feasible for examining cancers of the liver, kidney, and lymphoma given most studies presented risks for these sites in their published papers and these cancer sites are of interest given observations in the animal studies. Examination of site-specific cancers other than kidney cancer, liver cancer, and lymphoma, such as for childhood leukemia, is more difficult and not recommended due to few available high-quality studies. NRC (2006) specifically suggested EPA to:

1. Document essential design features, exposure, and results from the epidemiologic studies—Information on study design, exposure assessment approach, statistical analysis, and other aspects important to interpreting observations in a weight of evidence evaluation for individual studies is found in Section B.3. and site-specific estimated relative risks or measures of association are presented in Section 4;

2. Analyze the epidemiologic studies to discriminate the amount of exposure experience by the study population; exclude studies in meta-analysis based on objective criteria (e.g., studies in which it was unclear that the study population was exposed)—Appendix B.3. describes exposure assessment approach for

individual studies and inclusion criteria for identifying studies for meta-analysis are identified below;

3. Classify studies in terms of objective characteristics, such as on the basis of the study's design characteristics or documentation of exposure —Section B.3. groups studies by study design, analytical designs and geographic-based designs, with discussion of factors important to study design, endpoint measured, exposure assessment approach, study size, and statistical analysis methods including adjustment for potential confounding exposures;

4. Assess statistical power of each study—Table B.3 presents power calculations for cohort studies;

5. Combine case-control and cohort studies in the analysis, unless it introduces substantial heterogeneity—Appendix C discusses the meta-analysis statistical methods and findings;

6. Testing of heterogeneity (e.g., fixed or random effect models)—Appendix C discusses the meta-analysis statistical methods and findings;

7. Perform a sensitivity analysis in which each study is excluded from the analysis to determine whether any study significantly influences the finding—Appendix C discusses the meta-analysis statistical methods and findings.

Studies selected for inclusion in the meta-analysis met the following criteria: (1) cohort or case-control designs; (2) evaluation of incidence or mortality; (3) adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; (4) TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, job exposure matrices, water distribution models, or obtained from subjects using questionnaire (case-control studies); (5) relative risk estimates for kidney cancer, liver cancer, or lymphoma adjusted, at minimum, for possible confounding of age, sex, and race. Table B-5 in Section B.2.9.4 identifies studies included in the meta-analysis and studies that did not meet the inclusion criteria and the primary reasons for their deficiencies.

B.2.9.1. *Cohort Studies*

The cohort studies (Wilcosky et al., 1984; Shindell and Ulrich, 1985; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Sinks et al., 1992; Axelson et al., 1994; Greenland et al., 1994; Anttila et al., 1995; Henschler et al., 1995; Ritz, 1999a; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999, 2006a; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Chang et al., 2003, 2005; Zhao et al., 2005; Krishnadasan et al., 2007; Sung et al., 2007, 2008; Radican et al., 2008) with data on the incidence or morality of site-specific cancer in relation to trichloroethylene exposure range in size (803 [Hansen et al., 2001] to 86,868 [Chang et al., 2003, 2005]), and were conducted in Denmark, Sweden, Finland, Germany, Taiwan and the United States (see Table B-1). Three case-control studies nested within cohorts (Wilcosky et al., 1984; Greenland et al., 1994; Krishnadasan et al., 2007) are considered as cohort studies because the summary risk estimate from a nested case-control study, the odds ratio, was estimated from incidence density sampling and is considered an unbiased estimate of the hazard ratio, similar to a relative risk estimate from a cohort study. Two studies of deaths within a cohort were included in the group, but these studies lacked information on the person-year structure; i.e., both are proportionate mortality ratio studies, and did not satisfy the meta-analysis inclusion criteria for analytical study design (ATSDR, 2004; Clapp and Hoffman, 2008).

Cohort and nested case-control study designs are analytical epidemiologic studies and are generally relied on for identifying a causal association between human exposure and adverse health effects (U.S. EPA, 2005). Some subjects in the Hansen et al. study are also included in a study reported by Raaschou-Nielsen et al. (2003); however, any contribution from the former to the latter are minimal given the large differences in cohort sizes of these studies (Hansen et al., 2001; Raaschou-Nielson et al., 2003). Similarly, some females in Chang et al. (2003, 2005), a large cohort of 70,735 female and 16,133 male subjects, are included in Sung et al. (2007), a cohort of 63,982 female electronic workers from the same factory who were followed an additional 4-year period than subjects in Chang et al. (2003, 2005). Cancer observations for female subjects in these studies are considered as equivalent since they are derived from essentially the same population. Krishnadasan et al. (2007) is a nested case-control study of prostate cancer with cases and controls drawn from subjects in a large cohort of aerospace workers as subjects in Zhao et al. (2005), who did not report on prostate cancer, and met all the inclusion criteria except that for reporting a relative risk estimate for cancer of the kidney, liver or lymphoma.

Ten of the cohort studies met all five inclusion criteria: the cohorts of Blair et al. (1998) and its further follow-up by Radican et al. (2008), Morgan et al. (1998), Boice et al. (1999, 2006a), and Zhao et al. (2005) of aerospace workers or aircraft mechanics; Axelson et al. (1994), Anttila et al. (1995), Hansen et al. (2001), and Raaschou-Nielsen et al. (2003) of Nordic workers in multiple industries with TCE exposure; and Greenland et al. (1994) of electrical manufacturing workers. All ten cohort studies adopted statistical methods, e.g., life table analysis, Poisson regression analysis, or Cox Proportional Hazard analysis, that met epidemiologic standards, and were able to control for age, race, sex, and calendar time trends in cancer rates. Statistical analyses in Boice et al. (1999) adjusted for demographic variable such as age, race, and sex, and, also, included date of first employment and terminating date of employments, which may have decreased the statistical power of their analyses due to colinearity between age, first and last employment dates. Statistical analyses in Zhao et al. (2005) and Boice et al. (2006a) adjusted for potential effects by other occupational exposures on cancer and both Raaschou-Nielsen et al. (2003) and Zhao et al. (2005) examined possible confounding by smoking on TCE exposure and cancer risks using indirect approaches.

Of the ten studies, two studies reported risk estimates for both site-specific cancer incidence and mortality (Blair et al., 1998; its follow-up by Radican et al., (2008); Zhao et al., 2005), four studies reported risk estimates for cancer incidence only (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Krishnadasan et al., 2007) and three studies reported risk estimates for mortality only (Morgan et al., 1998; Boice et al., 1999, 2006a). Incidence ascertainment in two cohorts began 21 (Blair et al., 1998) and 38 years (Zhao et al., 2005) after the inception of the cohort. Specifically, Zhao et al. (2005) note "results may not accurately reflect the effects of carcinogenic exposure that resulted in nonfatal cancers before 1988." Because of the issues concerning case ascertainment raised by this incomplete coverage, incidence observations must be interpreted in light of possible bias reflecting incomplete ascertainment of incident cases. Furthermore, use of an internal referent population, nonexposed subjects drawn from the same or near-by facilities as exposed workers, in Blair et al. (1998) and Radican et al. (2008) for overall TCE exposure, and in Blair et al. (1998), Morgan et al. (1998), Boice et al. (1999), Zhao et al. (2005), Boice et al. (2006a), and Radican et al. (2008) for rank-ordered TCE exposure is expected to reduce bias associated with the healthy worker effect. Morgan et al. (1998) presents risk estimates for overall TCE exposure comparing mortality in their TCE subcohort to that expected using mortality rate of the U.S. population in an Environmental Health Strategies Final Report and sent to U.S. EPA by Paul Cammer, Ph.D., on behalf of the Trichloroethylene Issues Group (Environmental Health Strategies, 1997). The final report also contained risk estimates from internal analyses of rank-order TCE exposure and published as Morgan et al. (1998). Both internal cohort analyses of the rank-ordered exposure, presented in both the final report of Environment Health Strategies (1997) and Morgan et al. (1998), and overall TCE exposure, available in the final report or upon request, are based on the same group of internal referents, nonexposed TCE subjects employed at the same facility.

Subjects in these studies had a high likelihood or potential for TCE exposure, although estimated average exposure intensity for overall TCE exposure in some cohorts was considered as less than 10 or 20 ppm (time-weighted average). The exposure assessment techniques used in these cohort studies included a detailed job-exposure matrix (Greenland et al., 1994; Blair et al., 1998; its follow-up by Radican et al., 2008; Morgan et al., 1998; Boice et al., 1999, 2006a; Zhao et al., 2005; Radican et al. (2008), biomonitoring data (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001), or use of industrial hygiene data on TCE exposure patterns and factors that affect such exposure (Raaschou-Nielsen et al., 2003), with high probability of TCE exposure potential to individual subjects. The job-exposure matrix in six studies provided rank-ordered surrogate metrics for TCE exposure (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001; Blair et al., 1998 and its follow-up by Radican et al., 2008; Zhao et al., 2005), a strength compared to use of duration of employment as an exposure surrogate, e.g., Boice et al. (1999, 2006a) or Raachou-Nielsen et al. (2003), which is a poorer exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006). Rank-ordered TCE dose surrogates for low and medium exposure from the job-exposure matrix of Morgan et al. (1998) are uncertain because of a lack on information on frequency of exposure-related tasks and on temporal changes (NRC, 2006); only the high category for TCE exposure is unambiguous. The nested case-control study of Greenland et al. (1994) examined TCE as one of seven exposures and potential assigned to individual cases and controls using a job-exposure-matrix approach. However, the low exposure prevalence, missing job history information for 34% of eligible subjects, and study of pensioned workers only were other factors judged to lower this study's sensitivity for cancer hazard identification.

The remaining cohort studies (Wilcosky et al., 1984; Shindell and Ulrich, 1985; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Sinks et al., 1992; Henschler et al., 1995; Ritz, 1999a; Chang et al., 2003, 2005; Sung et al., 2007, 2008) less satisfactorily meet inclusion criteria. These studies, while not meeting the meta-analysis inclusion criteria, can inform the hazard analysis although their findings are weighted less than for observations in higher-quality studies, and observations may have alternative causes. Reasons for study insufficiencies varied. Nine studies do not assign TCE exposure potential to individual subjects (Shindell and Ulrich, 1985; Garabrant et al., 1988; Costa et al., 1989; Sinks et al., 1992; Chang et al., 2003, 2005; ATSDR, 2004; Sung et al., 2007, 2008; Clapp and Hoffman, 2008); all subjects are presumed as "exposed" because of employment in the plant or facility although individual subjects would be expected to have differing exposure potentials.

TCE exposure potential is ambiguous in both Wilcosky et al. (1984) and Ritz (1999a), two studies of low potential, low intensity TCE exposure compared to studies using exposure assessment approaches supported by information on job titles, tasks, and industrial hygiene monitoring data. Furthermore, high correlation in Ritz (1999a) between TCE and other exposures, particularly cutting fluids and radiation, may not have been sufficiently controlled in statistical analyses. Ritz et al. (1999a), furthermore, did not report estimated relative risks for kidney or lymphoma separately; rather, presenting relative risk estimates for kidney and bladder cancer combined and for all hemato- and lymphopoietic cancers.

Two studies do not sufficiently define the underlying cohort or there is uncertainty in cancer case or death ascertainment (Shindell and Ulrich, 1985; Henschler et al., 1995). Furthermore, magnitude of observed risk in Henschler et al. (1995), ATSDR (2004) and Clapp and Hoffman (2008) must be interpreted in a weight-of-evidence evaluation in light of possible bias introduced through use of analysis of proportion of deaths (proportionate mortality ratio) in ATSDR (2004) and Clapp and Hoffman (2008), or to inclusion of index kidney cancer cases in Henschler et al. (1995).

B.2.9.2. *Case-Control Studies*

Case-control studies on TCE exposure are of several site-specific cancers and include bladder (Siemiatycki, 1991; Siemiatycki et al., 1994; Pesch et al., 2000a); brain (Heineman et al., 1994; De Roos et al., 2001; childhood lymphoma or leukemia (Lowengart et al., 1987; McKinney et al., 1991; Shu et al., 1999, 2004; Costas et al., 2002); colon cancer (Siemiatycki, 1991; Goldberg et al., 2001); esophageal cancer (Siemiatycki, 1991; Parent et al., 2000a); liver cancer (Lee et al., 2003); lung (Siemiatycki, 1991), lymphoma (Hardell et al., 1994 [NHL, Hodgkin lymphoma]; Siemiatycki, 1991; Fritschi and Siemiatycki, 1996a; Nordstrom et al., 1998; [hairy cell leukemia]; Persson and Fredriksson, 1999 [NHL]; Miligi et al., 2006 [NHL and chronic lymphocytic leukemia (CLL)]; Seidler et al., 2007 [NHL, Hodgkin lymphoma]; Constantini et al., 2008 [leukemia types, CLL included in Miligi et al., 2006]; Wang et al., 2009 [NHL]); melanoma (Siemiatycki, 1991; Fritchi and Siemiatycki, 1996b); rectal cancer (Siemiatycki, 1991; Dumas et al., 2000); renal cell carcinoma, a form of kidney cancer (Siemiatycki, 1991; Parent et al., 2000b; Vamvakas et al., 1998; Dosemeci et al., 1999; Pesch et al., 2000b; Brüning et al., 2003; Charbotel et al., 2006, 2009); pancreatic cancer (Siemiatycki, 1991); and prostate cancer (Siemiatycki, 1991; Aronson et al., 1996). No case-control studies of reproductive cancers (breast or cervix) and TCE exposure were found in the peer-reviewed literature.

Several of the above publications are studies of cases and controls drawn from the same underlying population with a common control series. Miligi et al. (2006) and Costantini et al. (2008) presented observations from the Italian multicenter lymphoma population case-control study; Miligi et al. (2006) on occupation or specific solvent exposures and NHL, and who also included CLL and Hodgkin's lymphoma in the overall NHL category, and Costantini et al. (2008) who examined leukemia subtypes, and included CLL as a separate disease outcome. Pesch et al. (2000a, b), a multiple center population case- control study of urothelial cancers in

Germany, presented observations on TCE and bladder cancer, including cancer of the ureter and renal pelvis, in Pesch et al. (2000a) and renal cell carcinoma in Pesch et al. (2000b). Siemiatycki (1991), a case-control of occupational exposures and several site-specific cancers (bladder, colon, esophagus, lung, rectum, pancreas, and prostate) and designed to generate hypotheses about possible occupational carcinogens, presents risk estimates associated with TCE exposure using Mantel-Haentszel methods. Subsequent publications examine either TCE exposure (analyses of melanoma and colon cancers) or job title/occupation (all other cancer sites) using logistic regression methods (Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996a, b; Dumas et al., 2000; Parent et al., 2000a, b; Goldberg et al., 2001).

The population case-control studies with data on cancer incidence (Siemiatycki, 1991 [and related publications, Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996a, b; Dumas et al., 2000; Parent et al., 2000a, b; Goldberg et al., 2001]; Lowengart et al., 1987; McKinney et al., 1991; Hardell et al., 1994; Nordstrom et al., 1998; Vamvakas et al., 1998; Dosemeci et al., 1999; Kernan et al., 1999; Persson and Fredriksson, 1999; Pesch et al., 2000a, b; De Roos et al., 2001; Costas et al., 2002; Brüning et al., 2003; Shu et al., 2004; Charbotel et al., 2006, 2009; Miligi et al., 2006; Seidler et al., 2007; Constantini et al., 2008; Wang et al., 2009) or mortality (Heineman et al., 1994; Lee et al., 2003) in relation to trichloroethylene exposure range in size, from small studies with less than 100 cases and control (Costas et al., 2002) to multiple-center studies large-scale studies of over 2,000 cases and controls (Shu et al., 1999, 2004; Pesch et al., 2000a, b; Miligi et al., 2006; Costantini et al., 2008), and were conducted in Sweden, Germany, Italy, Taiwan, Canada and the United States (see Table B-2).

Thirteen of the case-control studies met the meta-analysis inclusion criteria identified in Section B.2.9 (Siemiatycki, 1991; Hardell et al., 1994; Nordstrom et al., 1998; Dosemeci et al., 1999; Persson and Fredriksson, 1999; Pesch et al., 2000 b; Brüning et al., 2003; Miligi et al., 2006; Charbotel et al., 2006, 2009; Seidler et al., 2007; Constantini et al., 2008, Wang et al., 2009). They were of analytical study design, cases and controls were considered to represent underlying populations and selected with minimal potential for bias; exposure assessment approaches included assignment of TCE exposure potential to individual subjects using information obtained from face-to- face, mailed, or telephone interviews; analyses methods were appropriate, well-documented, included adjustment for potential confounding exposures, with relative risk estimates and associated confidence intervals reported for kidney cancer, liver cancer or lymphoma.

All thirteen studies evaluated TCE exposure potential to individual cases and controls and a structured questionnaire sought information on self-reported occupational history and specific exposures such as TCE. Three studies assigned TCE exposure potential to cases and controls using self-reported information (Hardell et al., 1994; Nordstrom et al., 1998; Persson and Fredriksson, 1999) and two of these studies used judgment to assign potential exposure intensity (Nordstrom et al., 1998; Persson and Fredriksson, 1999). Persson and Fredriksson (1999) also assigned TCE exposure potential from both occupational and leisure use, the only study to do so. The ten other studies assigned TCE exposure potential using self-reported job title and occupational history, a superior approach compared to use of a job exposure matrix (JEM) supported by expert judgment and information on only self-reported information given its expect greater specificity (Siemiatycki, 1991; Dosemeci et al., 1999; Pesch et al., 2000b; Brüning et al., 2003; Miligi et al., 2006; Charbotel et al., 2006, 2009; Seidler et al., 2007; Constantini et al., 2008, Wang et al., 2009). Pesch et al. (2000b) assigned TCE exposure potential using both job exposure matrix and job-task exposure matrix (JTEM). The inclusion of task information is considered superior to exposure assignment using only job title since it likely reduces potential misclassification and, for this reason, relative risk estimates in Pesch et al. (2000b) for TCE from a JTEM are preferred. All studies except Hardell et al. (1994) and Dosemeci et al. (1999) developed a semiquantitative or quantitative TCE exposure surrogate.

These studies to varying degrees were considered as high-quality studies for weight-of evidence characterization of hazard. Both Brüning et al. (2003) and Charbotel et al. (2006, 2009) had *a priori* hypotheses for examining renal cell carcinoma and TCE exposure. Strengths of both studies are in their examination of populations with potential for high exposure intensity and in areas with high frequency of TCE usage and their assessment of TCE potential. An important feature of the exposure assessment approach of Charbotel et al. (2006) is their use of a large number of studies on biological monitoring of workers in the screw-cutting industry a predominant industry with documented TCE exposures as support. The other studies were either large multiple-center studies (Pesch et al., 2000a, b; Miligi et al., 2006; Constantini et al., 2008; Wang et al., 2009) or reporting from one location of a larger international study (Dosemeci et al., 1999; Seidler et al., 2007). In contrast to Brüning et al. (2003) and Charbotel et al. (2006, 2009), two studies conducted in geographical areas with widespread TCE usage and potential for exposure to higher intensity, a lower exposure prevalence to TCE is found [any TCE exposure: 15% of cases (Dosemeci et al., 1999); 6% of cases (Miligi et al., 2006); 13% of cases (Seidler et al., 2007); 13% of cases (Wang et al., 2008)] and most subjects identified as exposed to TCE probably had minimal contact [3% of cases with moderate/high TCE exposure (Miligi et al., 2006); 1% of cases with high cumulative TCE (Seidler et al., 2007); 2% of cases with high intensity, but of low probability TCE exposure (Wang et al., 2008)]. This pattern of lower exposure prevalence and intensity is common to community-based population case-control studies (Teschke et al., 2002).

Thirteen case-control studies did not meet specific inclusion criterion (Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996b; Dumas et al., 2000; Parent et al., 2000a; Goldberg et al., 2001; Vamvakas et al., 1998; Kernan et al., 1999; Shu et al., 1999, 2004; Pesch et al., 2000a; Costas et al., 2002; Lee et al., 2003). Vamvakas et al. (1998) has been subject of considerable controversy (Bloemen and Tomenson, 1995; Swaen, 1995; McLaughlin and Blot, 1997; Green and Lash, 1999; Cherrie et al., 2001; Mandel, 2001) with questions raised on potential for selection bias related to the study's controls. This study was deficient in the criterion for adequacy of case and control selection. Brüning et al. (2003), a study from the same region as Vamvakas et al. (1998), is considered a stronger study for identifying cancer hazard since it addresses many of the deficiencies of Vamvakas et al. (1998). Lee et al. (2003) in their study of hepatocellular cancer assigns one level of exposure to all subjects in a geographic area, and inherent measurement error and misclassification bias because not all subjects are exposed uniformly. Additionally, statistical analyses in this study did not control for hepatitis viral infection, a known risk factor for hepatocellular cancer and of high prevalence in the study area, Ten of twelve studies reported relative risk estimates for site-specific cancers other than kidney, liver, and lymphomas (Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996b; Kernan et al., 1999; Dumas et al., 2000; Parent et al., 2000a; Pesch et al., 2000a; Goldberg et al., 2001; Shu et al., 1999, 2004; Costas et al., 2002).

B.2.9.3. *Geographic-Based Studies*

The geographic-based studies (Isacson et al., 1985; AZ DHS, 1990, 1995; Aickin et al., 1992; Aickin, 2004; Mallin, 1990; Vartiainen et al., 1993; Cohn et al., 1994, Morgan and Cassady, 2002; ATSDR, 2006a, 2008) with data on cancer incidence (all studies) are correlation studies to examine cancer outcomes of residents living in communities with TCE and other chemicals detected in groundwater wells or in municipal drinking water supplies. These eight studies did not meet inclusion criteria and were deficient in a number of criteria.

All geographic-based studies are surveys of cancer rates for a defined time period among residents in geographic areas with TCE contamination in groundwater or drinking water supplies, or soil and are not of analytical designs such as cohort and case-control designs. A major shortcoming in all studies is, also, their low level of detail to individual subjects for TCE potential. The exposure surrogate is assigned to a community, town, or a geographically-defined area such as a contiguous grouping of census tracts as an aggregate level, typically based on limited number of water monitoring data from a recent time period and is a poor exposure surrogate because potential for TCE exposure can vary in these broad categories depending on job function, year, use of personal protection, and, for residential exposure, pollutant fate and transport, water system distribution characteristics, percent of time per day in residence, presence of mitigation devices, drinking water consumption rates, and showering times. Additionally, ATSDR (2008), the only geographic-based study to examine other possible risk factors on individual subjects, reported smoking patterns and occupational exposures may partly contribute to the observed elevated rates of kidney and renal pelvis cancer and lung cancer in subjects living in a community with contaminated groundwater and with TCE exposure potential from vapor intrusion into residences.

B.2.9.4. *Recommendation of Studies for Treatment Using Meta-Analysis Approaches*

All studies are initially considered for inclusion in the meta-analysis; however, as discussed through-out this section, some studies are better than others for inclusion in a quantitative examination of cancer and trichloroethylene. Studies included in the meta-analysis (statistical methods and findings discussed in Appendix C) met the following five inclusion criteria: (1) cohort or case-control designs; (2) evaluation of incidence or mortality; (3) adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; (4) TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, job exposure matrices, water distribution models, or obtained from subjects using questionnaire (case-control studies); (5) relative risk estimates for kidney cancer, liver cancer, or lymphoma adjusted, at minimum, for possible confounding of age, sex, and race. The twenty-three studies that met these inclusion are: Siemiatycki (1991), Axelson et al. (1994), Greenland et al. (1994), Hardell et al. (1994), Anttila et al. (1995), Blair et al. (1998), Morgan et al. (1998), Nordstrom et al. (1998), Dosemeci et al. (1999), Boice et al. (1999, 2006a), Persson and Fredriksson (1999), Pesch et al. (2000b), Hansen et al. (2001), Brüning et al. (2003), Raaschou-Nielsen et al. (2003), Zhao et al. (2005), Miligi et al. (2006), Charbotel et al. (2006, 2009), Seidler et al. (2007), Radican et al. (2008), and Wang et al. (2009). Table B-5 identifies studies included in the meta-analysis and studies that did not meet the inclusion criteria and the primary reasons for their deficiencies.

There is some overlap between the cohorts of Zhao et al. (2005) and Boice et al. (2006a), each cohort is identified from a population of workers, but these studies differ on cohort definition, cohort identification dates, disease outcome examined, and exposure assessment approach. Zhao et al. (2005) who adopted a semiquantitative approach for TCE exposure assessment is preferred to Boice et al. (2006a), whose TCE subcohort included subjects with a lower likelihood for TCE exposure and duration of exposure, a poor exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006).

Additionally, a larger number of site-specific cancer deaths identified with potential TCE exposure is observed by Zhao et al. (2005) compared to Boice et al. (2006a); e. g., 95 lung cancer cases with medium or high TCE exposure (Zhao et al., 2005) and 51 lung cancer cases with any TCE exposure (Boice et al., 2006a) (see further discussion in B.3.1.1.1.3.). Radican et al. (2008) studied the same subjects as Blair et al. (1998), adding an additional 10 years of follow-up and updating mortality. Observed site-specific cancer mortality risk estimates in Radican et al. (2008) did not change appreciably and were consistent with those reported in Blair et al. (1998) and is preferred. Blair et al. (1998) who also presented incidence relative risk estimates is recommended for inclusion in sensitivity analyses.

B.3. INDIVIDUAL STUDY REVIEWS AND ABSTRACTS

B.3.1. Cohort Studies

B.3.1.1. *Studies of Aerospace Workers*

Seven papers reported on cohort studies of aerospace or aircraft maintenance and manufacturing workers in large facilities.

B.3.1.1.1. ***Studies of Santa Susanna Field Laboratory workers.*** Trichloroethylene exposure to workers at Santa Susanna Field Laboratory (SSFL), an aerospace facility located nearby Los Angeles, California, operated by Rocketdyne/Atomics International, formerly a division of Boeing and currently owned by Pratt-Whitney, is subject of two research efforts: (1) the University of California at Los Angeles (UCLA) study, overseen by the California Department of Health Services and funded by the U.S. Department of Energy (DOE) (Morgenstern et al., 1997, 1999; Ritz et al., 1999) with two publications on trichloroethylene exposure and cancer incidence (Zhao et al., 2005; Krishnadasan et al., 2007) and mortality (Zhao et al., 2005); and, (2) the International Epidemiology Institute study (IEI), funded by Boeing after publication of the initial UCLA reports, of all Rocketdyne employees which included a mortality analysis of trichloroethylene exposure in a subcohort of SSFL test stand mechanics (Boice et al., 2006a). In addition to chemical exposure, both groups examine radiation exposure and cancer among Rocketdyne workers monitored for radiation (Ritz et al., 2000; Boice et al., 2006b).

Table B-5. Summary of rationale for study selection for meta-analysis

Decision Outcome	Studies	Primary reason(s)
Studies Recommended for Meta-analysis:		
	Siemiatycki, 1991; Axelson et al., 1994; Hardell, 1994; Greenland et al., 1994; Anttila et al., 1995; Morgan et al., 1998; Nordstrom et al., 1998; Boice et al., 1999, 2006a; Dosemeci et al., 1999; Persson and Fredriksson, 1999; Pesch et al., 2000b; Hansen et al., 2001; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Miligi et al., 2006; Seidler et al., 2007; Charbotel et al., 2006, 2009; Radican et al., 2008 [Blair et al., 1998, incidence]; Wang et al., 2009	Analytical study designs of cohort or case-control approaches; Evaluation of cancer incidence or cancer mortality; Specifically identified TCE exposure potential to individual study subjects by reference to industrial hygiene records, individual biomarkers, job exposure matrices, water distribution models, industrial hygiene data indicating a high probability of TCE use (cohort studies), or obtained information on TCE exposure from subjects using questionnaire (case-control studies); Reported results for kidney cancer, liver cancer, or lymphoma with relative risk estimates and corresponding confidence intervals (or information to allow calculation).
Studies Not Recommended for Meta-analysis:		
	ATSDR, 2004; Clapp and Hoffman, 2008 Cohn et al., 1994	Weakness with respect to analytical study design (i.e., geographic-based, ecological or proportional mortality ratio design)
	Wilcosky et al., 1984; Isacson et al., 1985; Shindell and Ulrich, 1985; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; AZ DHS, 1990, 1995; Mallin, 1990; Aickin et al., 1992; Sinks et al., 1992; Vartiainen et al., 1993; Morgan and Cassady, 2002; Lee et al., 2003; Aickin, 2004; Chang et al., 2003, 2005; Coyle et al., 2005; ATSDR, 2006a, 2008; Sung et al., 2007, 2008;	TCE exposure potential not assigned to individual subjects using job exposure matrix, individual biomarkers, water distribution models, or industrial hygiene data indicating a high probability of TCE use (cohort studies)
	Lowengart et al., 1987; Fredriksson et al., 1989; McKinney et al., 1991; Heineman et al., 1994; Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996b; Dumas et al., 2000; Kernan et al., 1999; Shu et al., 1999, 2004; Parent et al., 2000a; Pesch et al., 2000a; De Roos et al., 2001; Goldberg et al., 2001; Costas et al., 2002; Krishnadasan et al., 2007;	Cancer incidence or mortality reported for cancers other than kidney, liver, or lymphoma
	Ritz, 1999a	Subjects monitored for radiation exposure with likelihood for potential confounding; Cancer mortality and TCE exposure not reported for kidney cancer and all hemato- and lymphopoietic cancer reported as broad category
	Henschler et al., 1995	Incomplete identification of cohort and index kidney cancer cases included in case series
	Vamvakas et al., 1998	Control selection may not represent case series with potential for selection bias

B.3.1.1.1.1. ***<u>International epidemiology institute study of Rocketdyne workers.</u>***

B.3.1.1.1.1.1. *Boice et al. (2006a).*

B.3.1.1.1.1.1.1. <u>Author's abstract</u>.

> **Objective:** The objective of this study was to evaluate potential health risks associated with testing rocket engines. **Methods**: A retrospective cohort mortality study was conducted of 8372 Rocketdyne workers employed 1948 to 1999 at the Santa Susana Field Laboratory (SSFL). Standardized mortality ratios (SMRs) and 95% confidence intervals (CIs) were calculated for all workers, including those employed at specific test areas where particular fuels, solvents, and chemicals were used. Dose-response trends were evaluated using Cox proportional hazards models. **Results:** SMRs for all cancers were close to population expects among SSFL workers overall (SMR = 0.89; CI = 0.82-0.96) and test stand mechanics in particular (n = 1651; SMR = 1.00; CI = 0.86-1.1.6), including those likely exposure to hydrazines (n = 315; SMR = 1.09; CI = 0.75-1.52) or trichloroethylene (TCE) (n=1111; SMR = 1.00; CI = 0.83-1.19). Nonsignificant associations were seen between kidney cancer and TCE, lung cancer and hydrazines, and stomach cancer and years worked as a test stand mechanic. No trends over exposure categories were statistically significant. **Conclusion:** Work at the SSFL rocket engine test facility or as a test stand mechanic was not associated with a significant increase in cancer mortality overall or for any specific cancer.

B.3.1.1.1.1.1.2. <u>Study description and comment</u>. Boice et al. (2006a) examined all cause, all cancer and site-specific mortality in a subcohort of 1,651 male and female test stand mechanics who had been employed on or after 1949 to 1999, the end of follow-up, for at least 6 months at SSFL. Subjects were identified from 41,345 male and female Rocketdyne workers at SSFL (*n* = 8.372) and two nearby facilities (32,979). Of the 1,642 male test stand mechanics, 9 females were excluded due to few numbers, personnel listing in company phone directories were used to identify test stand assignments (and infer potential specific chemical exposures) for 1,440 subjects, and of this group, 1,111 male test stand mechanics were identified with potential trichloroethylene exposure either from the cleaning of rocket engines between tests or from more generalized use as a utility degreasing solvent. Cause-specific mortality is compared to several referents: (1) morality rates of the U.S. population, (2) mortality rates of California residents, (3) hourly nonadministrative workers at SSFL and two nearby facilities, and (4) 1,598 SSFL hourly workers; however, the published paper does not clearly present details of all analyses. For example, the referent population is not identified for the standardized mortality ratio (SMR) analysis of the 1,111 male subjects with TCE potential exposure and analyses examining exposure duration present point estimates and p-values from tests of linear trend, but not always confidence intervals (e.g., Boice et al. [2006a, Table 7] table footnotes).

Exposure assessment to trichloroethylene is qualitative without attempt to characterize exposure level as was done in the exposure assessment approach of Zhao et al. (2005) and Krishnadsen et al. (2007). Test stand mechanics were nonadministrative hourly positions and had the greatest potential for chemical exposures to TCE and hydrazine. Potential exposure to chemicals also existed for other subjects associated with test stand work such as instrument mechanics, inspectors, test stand engineers, and research engineers potential for chemical exposure, although Boice et al. (2006a) considered their exposure potential lower compared to that received by test stand mechanics and, thus, were not included in the cohort. Like that encountered by UCLA researchers, work history information in the personnel file was not specific to identify work location and test stand and Boice et al. (2006a) adopted ancillary information, company phone directories, as an aid to identify subjects with greater potential for TCE exposure. From these aids, investigators identified rocket stand assignment for 1,440 or 87% of the SSFL test stand mechanics. Bias is introduced through missing information on the other 211 subjects or if phone directories were not available for the full period of the study. Test stand mechanics, if exposed, had the likelihood for exposure to high TCE concentrations associated with flushing or cleaning of rocket engines; 593 of the 1,111 subjects (53%) were identified as having potential TCE exposure through rocket engine cleaning. The removal or flushing of hydrocarbon deposits in fuel jackets and in liquid oxygen dome of large engines entailed the use of 5 to 100 gallons of TCE, with TCE use starting around 1956 and ceased by the late 1960's at all test stands except one which continued until 1994. No information was provided on test stand and working conditions or the frequency of exposure-related tasks, and no atmospheric monitoring data were available on TCE. A small number of these subjects (121) also had potential exposure to hydrazines. The remaining 518 subjects in the TCE subcohort were presumed exposed to TCE as a utility solvent. Information on use of TCE as a utility solvent is lacking except that TCE as a utility solvent was discontinued in 1974 except at one test stand where it was used until 1984. These subjects have a lower likelihood of exposure compared to subjects with TCE exposure from cleaning rocket engines.

Several study design and analysis aspects limit this study for assessing risks associated with trichloroethylene exposure. Overall, exposures were likely substantially misclassified and their frequency likely low, particularly for subjects identified with TCE use as a utility solvent who comprise roughly 50% of the TCE subcohort. Analyses examining number of years employed at SSFL or worked as test stand mechanic as a surrogate for cumulative exposure has a large potential for misclassification bias due to the lack of air monitoring data and inability to account to temporal changes in TCE usage. Moreover, the exposure metric used in some dose-response analyses is weighted by the number of workers without rationale provided and would introduce bias if the workforce changed over the period covered by this study. Some information suggests this was likely (1) the number of cohort subjects entering the cohort decreased over the time period of this study, as much as a 20% decrease between 1960's and 1970s, and (2) ancillary information (http://www.thewednesdayreport.com/twr/twr48v7.htm, accessed March 11, 2008; DOE Closure Project, http://www.etec.energy.gov/Reading-Room/DeSoto.html, accessed March 11, 2008). Study investigators did not carry out exposure assessment for referents and no information is provided on potential trichloroethylene exposure. If referents had more than background exposure, likely for other hourly subjects with direct association with test stand work but with a job title other than test stand mechanic, the bias introduced leads to an underestimation of risk. TCE use at SSFL was widespread and rocket engine cleaning occurred at other locations besides at test sites (Morgenstern et al., 1999), locations from which the referent population arose.

Boice JD, Marano DE, Cohen SS, Mumma MT, Blott WJ, Brill AB, Fryzek JP, Henderson BE, McLaughlin JK. 2006a. Mortality among Rocketdyne workers who tested rocket engines, 1948-1999. J Occup Environ Med 48:1070−1092.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract “objective of this study was to evaluate potential health risks associated with testing rocket engines.”
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	54,384 Rocketdyne workers of which 41,351 were employed on or after 1-1-1948 and for at least 6 mos at Santa Susana Field Laboratory or nearby facilities. Of the 41,351 subjects, 1,651 were identified as having a job title of test stand mechanic and exposure assignments could be made for 1,440 of these subjects. Site-specific mortality rates of U.S. population and of all-other Rocketdyne employees. Potential TCE exposures of all other subjects (referents) not documented but investigators assumed referents are unexposed to TCE.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality from 1948 to 12-31-1999.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin’s lymphoma	Coding to ICD in use at time of death.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Qualitative exposure assessment, any TCE exposure. No quantitative information on TCE intensity by job title or to individual subjects or referents. Missing exposure potential to 12% of test stand mechanics; potential exposure hydrazine and/or TCE assigned to 1,440 of 1,651 test stand mechanics. Of 1,440 test stand mechanics, 1,111* identified with potential TCE exposure, 518 of the 1,111 identified as having presumed high intensity exposure from the cleaning of rocket engines. The remaining 593 subjects with potential exposure to TCE through use as “utility solvent,” a job task with low likelihood or potential for TCE exposure.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	0.4% for test stand mechanic cohort (1,651 subjects).
>50% cohort with full latency	35 years average follow-up; 88% of 1,651 test stand mechanics >20 yr follow-up.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE exposed subcohort—391 total deaths, 121 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	SMR analysis restricted to male hourly test stand mechanics using U.S. population rates as referent—no adjustment of potential confounders other than age and calendar-year. Cox proportional hazard models examining TCE exposure adjusted for birth year, year of hire and potential hydrazine exposure. Race was not included in Cox proportional hazard analysis.
Statistical methods	SMR analysis and Cox proportional hazard.
Exposure-response analysis presented in published paper	Duration of exposure (employment): 2-sided tests for linear trend.
Documentation of results	All analyses are not presented in published paper. Follow-up correspondence of C Scott, U.S. EPA, to J. Boice, of 12-31-06 and 02-28-07 remain unanswered as of November 15, 2007.

*Zhao et al. (2005), whose study period and base population overlaps that of Boice et al. (2006a), identified a larger number of subjects with potential TCE exposures; 2,689 subjects with TCE score > 3, a group having medium to high cumulative TCE exposure.

B.3.1.1.1.2. ***<u>University of California at Los Angeles (UCLA) studies of Rocketdyne workers.</u>***

B.3.1.1.1.2.1. *Krishnadasan et al. (2007).*

B.3.1.1.1.2.1.1. <u>Author's abstract</u>.

> **Background** To date, little is known about the potential contributions of occupational exposure to chemicals to the etiology of prostate cancer. Previous studies examining associations suffered from limitations including the reliance on mortality data and inadequate exposure assessment. **Methods** We conducted a nested case-control study of 362 cases and 1,805 matched controls to examine the association between occupational chemical exposures and prostate cancer incidence. Workers were employed between 1950 and 1992 at a nuclear energy and rocket engine-testing facility in Southern California. We obtained cancer incidence data from the California Cancer Registry and seven other state cancer registries. Data from company records were used to construct a job exposure matrix (JEM) for occupational exposures to hydrazine, trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs), benzene, and mineral oil. Associations between chemical exposures and prostate cancer incidence were assessed in conditional logistic regression models. **Results** With adjustment for occupational confounders, including socioeconomic status, occupational physical activity, and exposure to the other chemicals evaluated, the odds ratio for low/moderate TCE exposure was 1.3; 95%CI=0.8 to 2.1, and for high TCE exposure was 2.1; 95%CI=1.2 to 3.9. Furthermore, we noted a positive trend between increasing levels of TCE exposure and prostate cancer (p-value for trend=0.02). **Conclusion** Our results suggest that high levels of TCE exposure are associated with prostate cancer among workers in our study population.

B.3.1.1.1.2.2. *Zhao et al. (2005).*

B.3.1.1.1.2.2.1. <u>Author's abstract</u>.

> **Background** A retrospective cohort study of workers employed at a California aerospace company between 1950 and 1993 was conducted; it examined cancer mortality from exposures to the rocket fuel hydrazine. **Methods** In this study, we employed a job exposure matrix (JEM) to assess exposures to other known or suspected carcinogens—including trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs), mineral oils, and benzene—on cancer mortality (1960−2001) and incidence (1988–2000) in 6,107 male workers. We derived rate- (hazard-) ratios estimates from Cox proportional hazard models with time-dependent exposures. **Results** High levels of TCE exposure were positively associated with cancer incidence of the bladder (rate ratio (RR): 1.98, 95% confidence interval (CI) 0.93–4.22) and kidney (4.90; 1.23–19.6). High levels of exposure to mineral oils increased mortality and incidence of lung cancer (1.56; 1.02–2.39 and 1.99; 1.03–3.85), and incidence of melanoma (3.32; 1.20–9.24). Mineral oil exposures also contributed to incidence and mortality of esophageal and stomach cancers and of non-Hodgkin's lymphoma and leukemia when

> adjusting for other chemical exposures. Lagging exposure measures by 20 years changed effect estimates only minimally. No associations were observed for benzene or PAH exposures in this cohort. **Conclusions** Our findings suggest that these aerospace workers who were highly exposed to mineral oils experienced an increased risk of developing and/or dying from cancers of the lung, melanoma, and possibly from cancers of the esophagus and stomach and non-Hodgkin's lymphoma and leukemia. These results and the increases we observed for TCE and kidney cancers are consistent with findings of previous studies.

B.3.1.1.1.2.3. *Study description and comment*. The source population for Krishnadasen et al. (2007) and Zhao et al. (2005) is the UCLA chemical cohort of 6,044 male workers with 2 or more years of employment Rocketdyne between 1950 and 1993, who engaged in rocket testing at SSFL before 1980 and who have never been monitored for radiation. Zhao et al. (2005) examined cancer mortality between 1960−2001, an additional 7 years from earlier analyses of the chemical subcohort (Morgenstern et al., 1999; Ritz et al., 1999), and cancer incidence (5,049 subjects) between 1988−2000, matching cohort subjects to names in California's Cancer Registry and eight other state cancer registries. Deaths before 1998 are coded using ICD, 9th revision, and ICD-10 after this date; ICD-0 was used to code cancer incidence with leukemia, lymphoma, and other lymphopoietic tumors grouped on the basis of morphology codes. A total of 600 cancer deaths and 691 incident cancers were identified during the study period.

Krishnadasen et al. (2007) adopted a nested case-control design to examine occupational exposure to several chemicals and prostate cancer incidence in a cohort which included the SSFL chemically-exposed subjects and an additional 4,607 workers in the larger cohort who were enrolled in the company's radiation monitoring program. A total of 362 incident prostate cancers were identified between 1988 and 12-31-1999. Controls were randomly selected from the original cohorts using risk-set sampling and a 5:1 matching ratio on age at start of employment, age at diagnosis, and cohort.

Both studies are based on the same exposure assessment approach. Walk-through visits, interviews with managers and workers, job descriptions manual, and historical facility reports supported the development of a JEM with jobs ranked on a scale of 0 (no exposure) to 3 (highly exposure) on presumptive exposure reflecting relative intensity of that exposure over 3 temporal periods: 1950−1960, 1970s, 1980−1990. Of the 6,044 subjects, 2,689 had TCE exposure scores of >3 and 2,643 with an exposure score 3 or greater for hydrazine. Workers with job titles indicating technical or mechanical work on rocket engines were presumed to have high hydrazine rocket fuel exposure and high TCE exposure, which was used in cleaning rocket engines and parts. Although fewer subjects had exposure to benzene (819 subjects) or mineral oil (1,499 subjects), a high percentage of these subjects were also exposed to TCE. TCE use was widespread at the facility and other mechanics, maintenance and utility workers, and machinists were presumed as having exposure. No details were provided for job titles other than rocket test stand mechanics for assigning TCE exposure intensity and historical trends in TCE usage. Air monitoring data was absent for any chemicals prior to 1985 and investigators could not link study subjects to specific work locations and rocket-engine test stands. As a result, exposures were probably substantially misclassified, particularly those with low to moderate TCE exposure. Cumulative intensity score was the sum of the job-and time-specific intensity score and years in job. Exposure classification was assigned blinded to survival status and cause of death.

Proportional hazards modeling in calendar time with both fixed and time-depend predictors was used by Zhao et al. (2005) to estimate exposure effects on site-specific cancer incidence and mortality for a combined exposure group of medium and high exposure intensity with workers with no to low exposure intensity as referents. Variables in the proportional hazard model included time since first employment, socioeconomic status, age at diagnosis or death, and exposure to other chemical agents including benzene, polycyclic aromatic hydrocarbons (PAHs) mineral oil, and hydrazine. Krishnadasen et al. (2007) fit conditional logistic regression model to their data adjusting of cohort, age at diagnosis, occupation physical activity, socioeconomic status and all other chemical exposure levels. Both publications include exposure-response analysis and present p-values for linear trend. Race was not controlled in either study given the lack of recording on personnel records. Smoking histories was available for only a small percentage of the cohort; for those subjects reporting smoking information, mean cumulative TCE score did not differ between smokers and nonsmokers.

This study develops semiquantitative exposure levels and is strength of the exposure assessment. However, potential for exposure misclassification exists and would be of a nondifferential direction. Rocket engine test stand mechanics had likely exposure to TCE, kerosene, and hydrazine fuels; no information is available as to exposure concentrations. Statistical analyses in both Zhao et al. (2005) and Krishnadansan et al. (2007) present risk estimates for TCE that were adjusted for these other chemical exposures. Other strengths of this study include a long follow-up period for mortality, greater than an average time of 29 years of which 16 at SSFL, use of internal referents and the examination of cancer incidence, although under ascertainment of cases is likely given only 8 state cancer registries were used to identify cases and incidence ascertained after 1981, 40 years after the cohort's initial definition date.

Krishnadasan A, Kennedy N, Zhao Y, Morgenstern H, Ritz B. 2007. Nested case-control study of occupational chemical exposures and prostate cancer in aerospace and radiation workers. Am J Ind Med 50:383−390.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Nested case-control study of the UCLA chemical and radiation cohorts (Morgenstern et al., 1997, 1999) to assess occupational exposures including TCE and prostate cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	4,607 radiation cohort + 6,107 Santa Susana chemical cohort (Ritz et al., 1999; Zhao et al., 2005), excluded 1,410 deaths before 1988 (date of cancer incidence follow-up). Incident prostate cancer cases identified from eight State cancer registries (California, Nevada, Arizona, Texas, Washington Florida, Arkansas, and Oregon). Controls were randomly selected from the original cohorts using risk-set sampling. 362 cases and 1,805 controls (100% participation rate).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Prostate cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	TCE exposure assigned to cases and controls based on longest job held at company as identified from personnel records. Cumulative exposure—ranked exposure intensity score for TCE by 3 time periods—using method of Zhao et al. (2005). Blinded ranking of exposure status.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Employment records were used to assign exposure. 734 subjects (249 cases and 485 controls, or 33% of all cases and controls) were interviewed via telephone or sent a mailed questionnaire to obtain medical history, education and personal information on physical activity level and smoking history.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No proxy interviews.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Any TCE exposure: 135 cases (37%) and 668 controls (37%). High cumulative TCE exposure: 45 cases (12%) and 124 controls (7%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cohort, age at diagnosis, occupational physical activity, SES, other chemical exposures (benzene, PAHs, mineral oil, hydrazine). No adjustment for race due to lacking information; affect of race on OR examined using information from survey of workers still alive in 1999. Few African American workers (n = 7), TCE levels did not vary greatly with race.
Statistical methods	Crude and adjusted conditional logistic regression.
Exposure-response analysis presented in published paper	p-value for trend with exposure lag (0 yrs, 20 yr).
Documentation of results	Adequate.

OR=odds ratio. SES= socio-economic status.

Zhao Y, Krishnadasan A, Kennedy N, Morgenstern H, Ritz B. 2005. Estimated effects of solvents and mineral oils on cancer incidence and Mortality in a cohort of aerospace workers. Am J Ind Med 48:249−258.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From introduction "one aim of this new investigation was to determine whether these aerospace workers also developed cancers from exposures to other chemicals including trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs), mineral oils, and benzene."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	6,107 male workers employed for 2 or more years and before 1980 at Santa Susana Field Laboratory. Internal referents (no or low TCE exposure).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence between 1988−2000. Mortality between 1950−2001.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-0 for cancer incidence. Leukemia, lymphomas, and other lymphopoietic malignancies grouped on the basis of morphology codes. Mortality: ICD-9, before 1998, and ICD-10 thereafter. Incidence: ICD-Oncology Lymphoma and leukemia grouping includes lymphosarcoma and reticulosarcoma, Hodgkin's disease, other malignant neoplasm of the lymphoid and histiocytic tissue, multiple myeloma and immunoproliferative neoplasms, and all leukemias except chronic lymphoid leukemia. The following incident tumors were also included: Hodgkin's disease, leukemia, polycythemia vera, chronic myeloproliferative disease, myelosclerosis, eosinophilic conditions, platelet diseases, and red blood cell diseases.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Cumulative exposure—ranked exposure intensity score for TCE by 3 time periods Blinded ranking of exposure status.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	99% follow-up for mortality (6,044 of 6,107 subjects).
>50% cohort with full latency	Average latency = 29 yrs (Ritz et al., 1999).
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	600 cancer deaths, 621 cancer cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Time since first employment, SES, age (at incidence or mortality), exposure to other carcinogens, including hydrazine. No adjustment for race. Indirectly assessment of smoking through examination of smoking distribution by chemical exposure. Mean TCE cumulative exposure scores of smokers and nonsmokers is not statistically significant different.
Statistical methods	Cox proportional hazards modeling in calendar time with both fixed and time-dependent predictors. Exposure lagged 10 and 20 yrs.
Exposure-response analysis presented in published paper	Test for monotonic trend of cumulative exposure, two-sided p-value for trend.
Documentation of results	Liver cancer results are not reported in published paper.

SES = socio-economic status.

B.3.1.1.1.3. ***Comment on the Santa Susanna Field Laboratory (SSFL) studies***. Rocketdyne workers at SSFL are subject of two separate and independent studies. Both research groups draw subjects from the same underlying source population, Rocketdyne workers including those at SSFL, however, the methods adopted to identify study subjects and to define TCE exposure differ with each study. A subset of SSFL workers is common to both studies; however, no information exist in final published reports (Morgenstern et al., 1997, 1999; IEI, 2005) to indicate the percentage overlap between cohorts or between observed number of site-specific events.

Notable differences in both study design and analysis including cohort identification, endpoint, exposure assessment approaches, and statistical methods exist between Zhao et al. (2005) and Krishnadasan et al. (2007), whose source population is the UCLA cohort, and Boice et al. (2006a) whose source population is the IEI cohort. A perspective of each study's characteristics may be obtained from Table B-6, below.

Table B-6. Characteristics of epidemiologic investigations of Rocketdyne workers

Study	Boice et al. (2006a)	Zhao et al. (2005)
Source population	41,351 administrative/scientific and nonadministrative male and female employees between 1949−1999 at Rocketdyne SSFL and two nearby facilities	~55,000 subjects of SSFL and two nearby facilities employed between 1950 and 1993
TCE subcohort	1,111 male test stand mechanics with potential TCE exposure	6,107 males working at SSFL before 1980 and identified as test stand personnel, of whom 2,689 males had exposure scores greater than no- to low-TCE exposure potential
Pay-type (hourly)	100% of TCE subcohort	11.3%
Job title with potential TCE exposure	Test stand mechanics identified with greatest potential for TCE exposure Other job titles with direct association with test stand work—instrument mechanics, inspectors, test stand engineers, and research engineers—identified with lower exposure potential to TCE and included in referent population	High potential exposure group included job titles as propulsion/test mechanics or technicians; Medium potential exposure group included propulsion/test inspector, test or research engineer, and instrumentation mechanic; Low-exposure potential included employees who, according to job title may have been present during engine test firings but without direct contact
Exposure metric	Qualitative, yes/no, and employment duration	Cumulative exposure score = ∑ (exposure score (0−3) x number of years in job)
Endpoint	Mortality as of 1999	Mortality as of 2001 and Incidence as of 2000
Statistical analysis	Standardized mortality ratio Proportional hazards modeling with covariates for birth year, hire year, and potential exposure to hydrazine.	Proportional hazards modeling with covariates for time since first employment, socioeconomic status, age at event, and exposure to all other carcinogens, including hydrazine
Observed number of deaths:		
Total cancer	121	600
Lung	51	No/low, 99 Medium, 62 High, 33
Kidney	7	No/low, 7 Medium, 7 High, 3
Bladder	5	No/low, 8 Medium, 6 High, 3
NHL/Leukemia	6	No/low, 27 Medium, 27 High, 6

A number of strengths and limitations underlie these studies. First, the Zhao et al. (2005) and Krishnadasan et al. (2007) analyses is of a larger population and of more cancer cases or deaths; 600 cancer deaths and 691 cancer cases in Zhao et al. (2005) compared to 121 cancer deaths in the TCE subcohort of Boice et al. (2006a), and for prostatic cancer among all Rocketdyne workers, 362 incident prostatic cancer cases in Krishnandasan et al. (2007) compared to 193 deaths in Boice et al. (2006a). Second, exposed populations appear appropriately selected in the three studies although questions exist regarding the referent population in Boice et al. (2006a) whose referent population included subjects with some direct association with test stand work but whose job title was other than test stand mechanic. As a result, it appears that these studies identify TCE exposure potential different for possibly similar job titles. For example, jobs as instrument mechanics, inspectors, test stand engineers, and research engineers are identified with medium potential exposure in Zhao et al. (2005). Boice et al. (2006a) on the other hand included these subjects in the referent population and assumed they had background exposure. TCE use at SSFL was also widespread and rocket engine cleaning occurred at other locations besides at test sites (Morgenstern et al., 1999), locations from which the referent population in Boice et al. (2006a) arose. If referents in Boice et al. (2006a) had more than background exposure, the bias introduced leads to an underestimation of risk. Third, Zhao et al. (2005) and Krishnadasan et al. (2007) studies include an examination of incidence, and are likely to have a smaller bias associated with disease misclassification than Boice et al. (2006a) who examines only mortality. Fourth, use of cumulative exposure score although still subject to biases is preferred to qualitative approach for exposure assessment. Last, all three studies adjusted for potentially confounding factors such as smoking, socioeconomic status, and other carcinogenic exposures using different approaches either in the design of the study, such as Boice et al. (2006a) limitation to only hourly workers, or in the statistical analysis such as Zhao et al. (2005) and Krishnadansen et al. (2007). For this reason, the large difference in hourly workers between the UCLA cohort and Boice et al. (2006a) is not likely to greatly impact observations.

B.3.1.1.2. ***Blair et al. (1998), Radican et al. (2008).***

B.3.1.1.2.1. ***<u>Radican et al. (2008) abstract.</u>***

> OBJECTIVE: To extend follow-up of 14,455 workers from 1990 to 2000, and evaluate mortality risk from exposure to trichloroethylene (TCE) and other chemicals. METHODS: Multivariable Cox models were used to estimate relative risk (RR) for exposed versus unexposed workers based on previously developed exposure surrogates. RESULTS: Among TCE-exposed workers, there was no statistically significant increased risk of all-cause mortality (RR = 1.04) or death

from all cancers (RR = 1.03). Exposure-response gradients for TCE were relatively flat and did not materially change since 1990. Statistically significant excesses were found for several chemical exposure subgroups and causes and were generally consistent with the previous follow-up. CONCLUSIONS: Patterns of mortality have not changed substantially since 1990. Although positive associations with several cancers were observed, and are consistent with the published literature, interpretation is limited due to the small numbers of events for specific exposures.

B.3.1.1.2.2. ***Blair et al. (1998) abstract.***

OBJECTIVES: To extend the follow up of a cohort of 14,457 aircraft maintenance workers to the end of 1990 to evaluate cancer risks from potential exposure to trichloroethylene and other chemicals. **METHODS:** The cohort comprised civilians employed for at least one year between 1952 and 1956, of whom 5727 had died by 31 December 1990. Analyses compared the mortality of the cohort with the general population of Utah and the mortality and cancer incidence of exposed workers with those unexposed to chemicals, while adjusting for age, sex, and calendar time. **RESULTS:** In the combined follow up period (1952–90), mortality from all causes and all cancer was close to expected (standardized mortality ratios (SMRs) 97 and 96, respectively). Significant excesses occurred for ischemic heart disease (SMR 108), asthma (SMR 160), and cancer of the bone (SMR 227), whereas significant deficits occurred for cerebrovascular disease (SMR 88), accidents (SMR 70), and cancer of the central nervous system (SMR 64). Workers exposed to trichloroethylene showed non-significant excesses for non-Hodgkin's lymphoma (relative risk (RR) 2.0), and cancers of the oesophagus (RR 5.6), colon (RR 1.4), primary liver (RR 1.7), breast (RR 1.8), cervix (RR 1.8), kidney (RR 1.6), and bone (RR 2.1). None of these cancers showed an exposure-response gradient and RRs among workers exposed to other chemicals but not trichloroethylene often had RRs as large as workers exposed to trichloroethylene. Workers exposed to solvents other than trichloroethylene had slightly increased mortality from asthma, non-Hodgkin's lymphoma, multiple myeloma, and breast cancer. **CONCLUSION:** These findings do not strongly support a causal link with trichloroethylene because the associations were not significant, not clearly dose-related, and inconsistent between men and women. Because findings from experimental investigations and other epidemiological studies on solvents other than trichloroethylene provide some biological plausibility, the suggested links between these chemicals and non-Hodgkin's lymphoma, multiple myeloma, and breast cancer found here deserve further attention. Although this extended follow up cannot rule out a connection between exposures to solvents and some diseases, it seems clear that these workers have not experienced a major increase in cancer mortality or cancer incidence.

B.3.1.1.2.3. ***Study description and comment***. This historical cohort study of 14,457 (9,400 male and 3,138 female) civilian personnel employed at least one year between 1942 and 1956 at Hill Air Force Base in Utah examines mortality to the end of 1982 (Spirtas et al., 1991) to the end of 1990 (Blair et al., 1998), or to the end of 2000 (Radican et al., 2008). About half of the cohort was identified with exposure to TCE (6,153 white men and 1,051 white women). One-fourth of subjects were born before 1909 with an attained age of 43 years at cohort's identification date of 1952 and whose first exposure could have been as early as 1939, a cohort considered as a "survivor cohort."

As of December 2008, the end of follow-up in Radican et al. (2008), 8,580 deaths (3,628 in TCE subcohort) were identified, an increase of 2,853 deaths with the additional 8 years follow-up period compared to Blair et al. (1998) (5,727 total deaths, 2,813 among TCE subcohort subjects), with a larger proportion deaths among non-TCE exposed subjects (58%) as of December 2008 compared to the December 2000 (51%). Approximately 50% of TCE-exposed subjects and 60% of all cohort subjects had died, with mean age of 75 years for TCE-exposed subjects still alive and 45 or more years since the cohort's definition (1953 to 1955), a time period longer than that typically considered for an induction or latent window for detecting an adverse outcome like cancer. Blair et al. (1998) additionally examined cancer incidence among white TCE-exposed workers alive on 1-1-1973, a period of 31 years after the cohort's inception date, to the end of 1990. Incident cancer cases are likely under ascertained for this reason.

Statistical analyses in Spirtas et al. (1991) and Blair et al. (1998) focus on site-specific mortality for white subjects or subjects with unknown race who were assumed to as white since 97% of all subjects with know race were white. SMRs are presented with expected numbers of deaths based upon age-, race- and year-specific mortality rates of the Utah population (Spirtas et al., 1991; Blair et al., 1998) or rate ratios for mortality or cancer incidence for the TCE subcohort from Poisson regression models, adjusting for date of birth, calendar year of death, and sex where appropriate, and an internal standard of mortality rates of the cohort's nonchemical exposed subjects (internal referents) (Blair et al., 1998). Blair et al. (1998), in addition to their presentation in the published papers of risk estimates associated with TCE exposure, also, presented risk estimates for subjects with an aggregated category of "any solvent exposure" (ever exposed) and for exposure to 14 solvents. To compare with risk ratios from Poisson regression models of Blair et al. (1998), Radican et al. (2008) adopted Cox proportional hazard models to reanalyze mortality observations of follow-up through 1990. For most site-specific cancers, Radican et al. (2008) did not observe large differences between the Cox hazard ratio and Poisson rate ratio of Blair et al. (1998), although difference between risk estimates from Cox proportional hazard and Poisson regression of 20% or larger was observed for kidney cancer (increased risk estimate) and primary liver cancer (decreased risk estimate). Radican et al. (2008), furthermore, noted hazard ratios for all subjects were similar to results for white subjects only; therefore, their analyses of follow-up through 2000 included all subjects.

The original exposure assessment of Stewart et al. (1991) who conducted a detailed exposure assessment of TCE exposures at Hill Air Force Base was used by Radican et al. (2008), Blair et al. (1999), and Spirtas et al. (1991). Their was limited for linking subjects with exposures principally because solvent exposures were associated with work in "shops," but work records listed only broad job titles and administrative units. As a result, exposures were probably substantially misclassified, particularly in "mixed solvent group." Trichloroethylene was used principally for degreasing and hand cleaning in work areas during 1955-1968. TCE was the predominant solvent used in the few available vapor degreasers located in the electroplating (main hanger), propeller, and engine repair shops before the mid-1950 and, afterwards, as a cold state solvent, replacing Stoddard solvent. Solvents, notably TCE after 1955, were used primarily by aircraft mechanics with short but high exposures and sheet metal workers for spot clean aircraft surfaces. The investigators determined that 32% had "frequent" exposures to peak concentrations (one or two daily peaks of about 15 minutes to trichloroethylene at 200-600 ppm) during vapor degreasing. Work areas were located in very large buildings with few internal partitions, which aided dispersion of trichloroethylene. While TCE exposures were less controlled in the 1950s, by the end of 1960s, TCE exposure had been reduced significantly. Only a small number of subjects with "high" exposure had long-duration exposures, no more than 16%. Few workers were exposed only to trichloroethylene; most had mixed exposures to other chlorinated and nonchlorinated solvents. Person-years of exposure were computed from date of first exposure, which could have been as early as 1939, to the end of 1982.

Overall, Blair et al. (1998) and Radican et al. (2008) are high quality studies with approximately half of the larger cohort identified as having some potential for TCE exposure (the TCE subcohort) and calculation of cancer risk estimates for TCE exposure, either risk ratios in Blair et al. (1998) or hazard ratios in Radican et al. (2008), using workers in the cohort without any chemical exposures as referent population, superior to standardized mortality ratios of Spirtas et al. (1991) who first reported on mortality and TCE exposure. Use of an internal referent population of workers from the same company or plant, but lacking the exposure of interest, is considered to reduce bias associated with the healthy worker effect. For follow-up in Radican et al. (2008) who examined mortality 45 years after first exposure and likely at the tail of or beyond a window for cancer induction time, any influence on exposure on disease development or detection times would be diminshed or less evident if exposures like TCE shortened induction time, e.g., if exposure shortened the natural course of disease development, which would become evident in an unexposed subjects with longer follow-up periods. The induction time of 35 years in Blair et al. (1998) may also fall outside a cancer induction window; however, it is more consistent with cancer induction times observed with other chemical carcinogens such as aromatic amines (Weistenhöfer et al., 2008) and vinyl chloride (Du and Wang, 1998). A strong exposure assessment was performed, but precision in the exposure assignment was limited by vague personnel data. The cohort had a modest number of highly exposed (about 100 ppm) subjects, but overall most were exposed to low concentrations (about 10 ppm) of trichloroethylene.

Radican L, Blair A, Stewart P, Wartenberg D. 2008. Mortality of aircraft maintenance workers exposed to trichloroethylene and other hydrocarbons and chemicals: extended follow-up. J Occup Environ Med 50:1306-1319.

Blair A, Hartge P, Stewart PA, McAdams M, Lubin J. 1998. Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: extended follow-up. Occup Environ Med 55:161−171.

Spirtas R, Stewart PA, Lee JS, Marano DE, Forbes CD, Grauman DJ, Pettigrew HM, Blair A, Hoover RN, Cohen JL. 1991. Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. Br J Ind Med 48:515-530.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Abstract: "...to evaluate cancer risks from potential exposure to trichloroethylene and other chemicals."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	All civilians employed at Hill AFB for ≥1 yr between 1-1-1952 and 12-31-1956; cohort of 14,457 workers identified form earnings records. TCE subcohort—7,204 white males and females (50%). External referents, all civilian cohort—Utah population rates, 1953−1990. Internal referents, TCE subcohort analysis of mortality (Blair et al., 1998; Radican et al., 2008) and incidence (Blair et al., 1998)—workers without chemical exposures.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality, all civilian cohort and TCE subcohort. Incidence, TCE subcohort.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Underlying and contributing causes of deaths as coded to ICDA 8.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Detailed records on setting and job activities, worker interviews; work done in large open shops; shops not recorded in personnel records, link of job with IH data was weak. Limited exposure IH measurements for TCE between 1960−1990. Plant JEM, rank order assignments by history; determined exposure duration during vapor degreasing tasks about 2,000 ppm-h and hard degreasing about 20 ppm-h. **Median exposure were about 10 ppm for rag and bucket (cold degreasing process); 100−200 ppm for vapor degreasing (Stewart et al., 1991). Cherrie et al. (2001) estimated long-term exposure as ~50 ppm with short-term excursion up to ~600 ppm. NRC (2006) concluded the cohort had a modest number of highly exposed (about 100 ppm) subjects, but overall most were exposed to low TCE concentrations (about 10 ppm).**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	97% of cohort traced successfully to 12-31-1982.
>50% cohort with full latency	Yes, all subjects followed minimum of 35 yrs (Blair et al., 1998) or 45 yrs (Radican et al., 2008).
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE subcohort—2,813 deaths (39%), 528 cancer deaths, and 549 incident cancers (1973-1990) (Blair et al., 1998); 3,628 deaths (50%). 729 cancer deaths (Radican et al., 2008).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	SMR analysis evaluates age, sex, and calendar year (Spirtas et al., 1991). Date of hire, calendar year of death, and sex in Poisson regression analysis (Blair et al., 1998). Age, gender, and race (to compare with RR of Blair et al.,[1998], or age and gender for follow-up to 2000] in Cox proportional hazard analysis (Radican et al., 2008).
Statistical methods	External analysis is restricted to Caucasian subjects—Life table analysis for mortality (Spirtas et al., 1991). Internal analysis restricted to Caucasian subjects or subject of unknown race assumed to be Caucasian and followed to 1990—Poisson regression (Blair et al., 1998) or Cox Proportional Hazard (Radican et al., 2008). Internal analysis—all subjects followed to 2000 (Radican et al., 2008).
Exposure-response analysis presented in published paper	Risk ratios from Poisson regression model and hazard ratios from Cox Proportional Hazard model for exposure rankings but no formal statistical trend test presented in papers.
Documentation of results	Adequate.

RR = relative risk.

B.3.1.1.3. *Boice et al. (1999).*

B.3.1.1.3.1. ***Author's abstract.***

OBJECTIVES: To evaluate the risk of cancer and other diseases among workers engaged in aircraft manufacturing and potentially exposed to compounds containing chromate, trichloroethylene (TCE), perchloroethylene (PCE), and mixed solvents. **METHODS:** A retrospective cohort mortality study was conducted of workers employed for at least 1 year at a large aircraft manufacturing facility in California on or after 1 January 1960. The mortality experience of these workers was determined by examination of national, state, and company records to the end of 1996. Standardized mortality ratios (SMRs) were evaluated comparing the observed numbers of deaths among workers with those expected in the general population adjusting for age, sex, race, and calendar year. The SMRs for 40 causes of death categories were computed for the total cohort and for subgroups defined by sex, race, and position in the factory, work duration, year of first employment, latency, and broad occupational groups. Factory job titles were classified as to likely use of chemicals, and internal Poisson regression analyses were used to compute mortality risk ratios for categories of years of exposure to chromate, TCE, PCE, and mixed solvents, with unexposed factory workers serving as referents. **RESULTS:** The study cohort comprised 77,965 workers who accrued nearly 1.9 million person-years of follow up (mean 24.2 years). Mortality follow-up, estimated as 99% complete, showed that 20,236 workers had died by 31 December 1996, with cause of death obtained for 98%. Workers experienced low overall mortality (all causes of death SMR 0.83) and low cancer mortality (SMR 0.90). No significant increases in risk were found for any of the 40 specific causes of death categories, whereas for several causes the numbers of deaths were significantly below expectation. Analyses by occupational group and specific job titles showed no remarkable mortality patterns. Factory workers estimated to have been routinely exposed to chromate were not at increased risk of total cancer (SMR 0.93) or of lung cancer (SMR 1.02). Workers routinely exposed to TCE, PCE, or a mixture of solvents also were not at increased risk of total cancer (SMRs 0.86, 1.07, and 0.89, respectively), and the numbers of deaths for specific cancer sites were close to expected values. Slight to moderately increased rates of non-Hodgkin's lymphoma were found among workers exposed to TCE or PCE, but none was significant. A significant increase in testicular cancer was found among those with exposure to mixed solvents, but the excess was based on only six deaths and could not be linked to any particular solvent or job activity. Internal cohort analyses showed no significant trends of increased risk for any cancer with increasing years of exposure to chromate or solvents.

The results from this large scale cohort study of workers followed up for over 3 decades provide no clear evidence that occupational exposures at the aircraft manufacturing factory resulted in increases in the risk of death from cancer or other diseases. Our findings support previous studies of aircraft workers in which cancer risks were generally at or below expected levels.

B.3.1.1.3.2. ***Study description and comment***. This study was conducted on an aircraft manufacturing worker cohort employed at Lockheed-Martin in Burbank, California with exposure assessment described by Marano et al. (2000). This large cohort study of 77,965 subject workers with at least 1 year employment on or after 1-1-1960, examined causes of mortality in the entire cohort, but also by broad job titles and for selected chemical exposures including TCE. Mortality was assessed as of 12-31-1996, with subjects lacking death certificates presumed alive at end of follow-up. Exposure assessment developed using a method of exposure assignment by job categories based on job histories (Kardex cards) and the judgment of long-term employees. Job histories were not available for every worker, and, if missing, auxiliary sources of job information were used to broadly classify workers into various job categories. Only subjects with job histories as recorded on Kardex cards are included in exposure duration analyses. TCE was used for vapor degreasing on routine basis prior to 1966 and, given the cohort beginning date of 1960, only a small percentage of the total cohort was identified as having potential TCE exposure. The investigators determined that 5,443 factory workers had potential TCE exposure. Of these subjects, 3% (2,267 out of 77,965 subjects) had "routine" defined as use of TCE as part of daily job activities and an additional 3,176 subjects (4%) had potential "intermittent" based upon job title and judgment of nonroutine or nondaily TCE usage and were included in the mortality analysis. No information was provided on building and working conditions or the frequency of exposure-related tasks, and no atmospheric monitoring data were available on TCE, although some limited data were available after 1970 on other solvents such as perchloroethylene, which replaced TCE in 1966 in vapor degreasing, methylene chloride, and 1,1,1-trichloroethane. Without more information, it is not possible to determine the quality of some of the TCE assignments. This study had limited ability to detect exposure-related effects given its use of duration of exposure, a poor exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006). Lacking monitoring information, analyses examining the number of years of routine and intermittent TCE exposure are likely biased due to exposure misclassification related to inability to account for changes in process and chemical usage patterns over time. Stewart et al. (1991) show atmospheric TCE concentrations decreased over time. Similarly, an observation of inverse relationship between some site-specific causes of death and duration of exposure may be due to selection bias or to misallocation of person-years of follow-up (NYS DOH, 2006).

Boice JD, Marano DE, Fryzek JP, Sadler CJ, McLaughlin JK. 1999. Mortality among aircraft manufacturing workers. Occup Environ Med 56:581−597.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract: “To evaluate the risk of cancer and other diseases among workers engaged in aircraft manufacturing and potentially exposed to compounds containing chromate, trichloroethylene (TCE), perchloroethylene (PCE), and mixed solvents.”
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	All workers employed on or after 1-1-1960 for at least 1 yr at Lockheed Martin aircraft manufacturing factories in California. Control population: U.S. mortality rates or factory workers no exposed to any solvent (internal referents).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin’s lymphoma	ICD code in use at the time of death.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Qualitative. Few exposure measurements existed prior to the late 1970s, a period after TCE had been discontinued at Lockheed-Martin aircraft manufacturing factories. Subjects are categorized as potentially TCE exposed received on a routine basis (2,075 subjects), daily job activity, or routine and intermittent basis (3,016 subjects), nonroutine or nondaily TCE usage, based on information on Service Record and Permanent Employment Record (Kardex) and other sources of job history information for subjects lacking Kardex cards.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	This study does not adopt methods to verify vital status of employees. All workers for which death certificate were not found are assumed to be alive until end of follow-up.
>50% cohort with full latency	Average follow-up of TCE cohort was 29 yrs.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,100 total deaths and 277 cancer deaths in TCE subcohort.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	SMR analysis—age, sex and calendar-time. Poisson regression using internal referents—birth date, date first employed, date of finishing employment, race, and sex.
Statistical methods	SMR for routine TCE exposure subcohort. Poisson regression for routine and intermittent TCE exposure subcohort.
Exposure-response analysis presented in published paper	Duration of exposure for subjects with Kardex cards only— 2-sides test for linear trend.
Documentation of results	Adequate.

B.3.1.1.4. *Morgan et al. (1998, 2000).*

B.3.1.1.4.1. ***Author's abstract.***

> We measured mortality rates in a cohort of 20,508 aerospace workers who were followed up over the period 1950-1993. A total of 4,733 workers had occupational exposure to trichloroethylene. In addition, trichloroethylene was present in some of the washing and drinking water used at the work site. We developed a job-exposure matrix to classify all jobs by trichloroethylene exposure levels into four categories ranging from "none" to "high" exposure. We calculated standardized mortality ratios for the entire cohort and the trichloroethylene exposed subcohort. In the standardized mortality ratio analyses, we observed a consistent elevation for nonmalignant respiratory disease, which we attribute primarily to the higher background rates of respiratory disease in this region. We also compared trichloroethylene-exposed workers with workers in the "low" and "none" exposure categories. Mortality rate ratios for nonmalignant respiratory disease were near or less than 1.00 for trichloroethylene exposure groups. We observed elevated rare ratios for ovarian cancer among those with peak exposure at medium and high levels] relative risk (RR) = 2.74; 95% confidence interval (CI) = 0.84-8.99] and among women with high cumulative exposure (RR = 7.09; 95% CI = 2.14-23.54). Among those with peak exposures at medium and high levels, we observed slightly elevated rate ratios for cancers of the kidney (RR = 1.89; 95% CI = 0.85-4.23), bladder (RR = 1.41; 95% CI = 0.52-3.81), and prostate (RR = 1.47; 95% CI = 0.85-2.55). Our findings do not indicate an association between trichloroethylene exposure and respiratory cancer, liver cancer, leukemia or lymphoma, or all cancers combined.

Erratum:

> One of the authors of the article entitled Mortality of aerospace workers exposed to trichloroethylene, by Robert W. Morgan, Michael A. Kelsh, Ke Zhao, and Shirley Heringer, published in Epidemiology 1998;9:424-431, informed us of some errors in one of the tables. In Table 5, the authors had inadvertently included both genders in counting person-years, rather than presenting gender-specific risk ratios for prostate and ovarian cancer. In addition, one subject, in the high trichloroethylene (TCE) exposure category, had been incorrectly classified with a diagnosis of ovarian cancer, instead of other female genital cancer. The authors report that correction of these errors did not change the overall conclusions of the study. The correct estimates of effect for prostate and ovarian cancer are presented in the Table below.

B.3.1.1.4.2. *Study description and comment*. This study of a cohort of 20,508 aircraft manufacturing workers employed for at least 6 months between 1950 and 1985 at Hughes Aircraft in Arizona was followed through 1993 for mortality. Cause-specific SMRs are resented for the entire cohort and the TCE-subcohort using U.S. Mortality rates from 1950−1992 as referents. Additionally, internal cohort analyses fitting Cox proportional hazards models are presented comparing risks for those with TCE exposure to never-exposed subjects. Morgan et al. (1998, 2000) do not identify job titles of individuals in the never-exposed group; however, it is assumed these individuals were likely white-collar workers, administrative staff, or other blue-collar worker with chemical or solvents exposures other than TCE.

The company conducted a limited semiquantitative assessment of TCE exposure based on the judgment of long-term employees. Most TCE exposure occurred in vapor degreasing units between 1952 and 1977. No details were provided on the protocol for processing the jobs in the work histories into job classifications; no examples were provided. Additionally, no information is provided other chemical exposures that may also have been used in the different jobs. Of the 20,508 subjects, 4,733 were identified with TCE exposure. Exposure categories were assigned to job classifications: high = worked on degreasers (industrial hygiene reported exposures were >50 ppm); medium = worked near degreasers; and low = work location was away from degreasers but "occasional contact with (trichloroethylene)." There was also a "no exposure" category. No data were provided on the frequency of exposure-related tasks. Without more information, it is not possible to determine the quality of some of these assignments. Only the high category is an unambiguous setting. Depending on how the degreasers were operated, operator exposure to trichloroethylene might have been substantially greater than 50 ppm. Furthermore, TCE intensity likely changed over time with changes in degreaser operations and exposure assignment based on job title only is able to correctly place subjects with a similar job title but held at different time periods. Furthermore, there are too many possible situations in which an exposure category of medium or low might be assigned to determine whether the ranking is useful. Therefore, the medium and low rankings are likely to be highly misclassified. Deficiencies in job rankings are further magnified in the cumulative exposure groupings. Internal analyses examine TCE exposed, defined as low and high cumulative exposure, compared to never-TCE exposed subjects. Low cumulative exposure group includes any workers with the equivalent of up to 5 years of exposure at jobs at low exposure or 1.4 years of medium exposure; all other workers were placed in the high cumulative exposure grouping. Ambiguity in low and medium job rankings and the lack of exposure data to define "medium" and "low" precludes meaningful analysis of cumulative exposure, specifically, and exposure-response, generally.

The development of exposure assignments in this study was insufficient to define exposures of the cohort and bias related to exposure misclassification is likely great. The inability to account for changes in TCE use and exposure potential over time introduces bias and may dampen observed risks. This study had limited ability to detect exposure-related effects and, overall, limited ability to provide insight on TCE exposure and cancer outcomes.

Morgan RW, Kelsh MA, Zhao K, Heringer S. 1998. Mortality of aerospace workers exposure to trichloroethylene. Epidemiol 9:424−431.

Morgan RW, Kelsh MA, Zhao K, Heringer S. 2000. Mortality of aerospace workers exposed to trichloroethylene. Erratum. Epidemiology 9:424−431.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	"measured mortality rates in a cohort of aerospace workers, comparing TCE workers with workers in low and none exposure categories."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	20,508 male and female workers are identified using company records and who were employed at plant for at least 6 mos between 1-1-1950 and 12-31-1985. TCE subcohort—4,733 (23%) male and female subjects. External referents—U.S. population rates, 1950−1992. Internal referents—Analysis of peak exposure, Low or no TCE exposure; analysis of cumulative exposure, never exposed to TCE. Internal referents are likely white-collar workers, administrative staff, and blue-collar workers with chemical exposure other than TCE. White-collar and administrative staff subjects are not representative of blue-collar workers due to SES and sex differences. Also, the never-TCE exposed blue-collar workers may potentially have other chlorinated solvents exposures, exposures that may be associated with a similar array of targets as TCE. These individuals may not be representative of a nonchemical exposed population as that used in Blair et al. (1998).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	No, ICD in use at time of death (ICD 7, 8, 9).

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Semiquantitative. Limited IH measurements before 1975. Jobs ranked into high, medium, or low intensity exposure categories; categories are undefined as to TCE intensity. **Jobs with high intensity exposure rating involved work on degreaser machines with TCE exposure equivalent to 50 ppm**; assigned exposure score of 9. Job with medium rating were near (distance undefined in published paper) degreasing area and a score of 4. Jobs with low rating were away (undefined distance) from degreasing area and assigned score of 1. Cumulative exposure score = $\sum$ (duration exposure × score). Peak exposure defined by job with highest ranking score.
CATEGORY D: FOLLOW-UP (Cohort)	
More than 10% loss to follow-up	No, 27 subjects were excluded from analysis due to missing information.
>50% cohort with full latency	Average 22 yrs of follow-up for TCE subcohort.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE subcohort—917 total deaths (19%) of subcohort, 270 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, race, sex, and calendar year in SMR analysis. Internal analysis- age (for bladder, prostate, ovarian cancers) and, age and sex (liver, kidney cancers).
Statistical methods	Life table analysis (SMR). Cox proportional hazards modeling (unexposed subjects as internal referents)—peak and two-levels of cumulative exposure (Environmental Health Strategies, 1997; Morgan et al., 1998); any TCE exposure (Environmental Health Strategies, 1997).

Exposure-response analysis presented in published paper	Qualitative presentation, only; no formal statistical test for linear trend.
Documentation of results	Adequate.

SES = socio-economic status.

B.3.1.1.5. *Costa et al. (1989).*

B.3.1.1.5.1. ***Author's abstract.***

> Mortality in a cohort of 8626 workers employed between 1954 and 1981 in an aircraft manufacturing factory in northern Italy was studied. Total follow up was 132,042 person-years, with 76% accumulated in the age range 15 to 54. Median duration of follow up from the date of first employment was 16 years. Vital status was ascertained for 98.5% of the cohort. Standardized mortality ratios were calculated based on Italian national mortality rates. Altogether 685 deaths occurred (SMR = 85). There was a significant excess of mortality for melanoma (6 cases, SMR = 561). Six deaths certified as due to pleural tumors occurred. No significant excess of mortality was found in specific jobs or work areas.

B.3.1.1.5.2. *Study description and comment*. This study assesses mortality in a small cohort of 8,626 aircraft manufacturing workers employed between 1954 and the end of follow-up in June, 1981. A period of minimum employment duration before accumulating person-years was not a prerequisite for cohort definition. The cohort included employees identified as blue collar workers, technical staff, administrative clerks, and white-collar workers. Blue-collar workers comprised 7,105 of the 8,626 cohort subjects. Mortality was examined for all workers and included job title of blue collar workers, technical staff members, administrative clerks, and white collar workers- not otherwise specified. No exposure assessment was used and the published paper does not identify chemical exposures. In fact, Costa et al. (1989) do not even mention TCE in the paper.

Overall, the lack of exposure assessment, the inability to identify TCE as an exposure to this cohort, and the inclusion of subjects who likely do not have potential TCE exposure are reasons why this study is not useful for determining whether trichloroethylene may cause increased risk of disease.

Costas G, Merletti F, Segnan N. 1989. A mortality study in a north Italian aircraft factory. Br J Ind Med 46:738−743.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The 1st paragraph of the paper identified this study was carried out to investigate an apparently high number of malignant tumors among employees that were brought to the attention of the local health authority by staff representative. This study was not designed to examine TCE exposure and cancer outcomes.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort is defined as all workers every employed between 1-1-1954 and 6-30-1981 (end of follow-up) at a north Italian aircraft manufacturing factory. Cohort include 8.626 subjects: 950 women (636 clerks, 314 blue-collar workers/technical staff) and 7,676 men (5,625 blue collar workers, 965 technical staff, 571 administrative clerks, and 515 white collar workers). External referent—Age, year (5-yr periods over 1955−1981)-sex and cause-specific death rates of Italian population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Causes and underlying causes of death coded to ICD rule in effect at the time of death and grouped into categories consistent with ICD 8th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure is defined as employment in the factory. TCE is not mentioned in published paper and no exposure assessment was carried out by study investigators.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Vital status ascertained for 98% of cohort; 2% could not be traced (1% unknown and 1% had emigrated).
>50% cohort with full latency	Average mean follow-up: males, 17 yrs; females, 13 yrs.

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	642 total deaths, 168 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex and calendar year.
Statistical methods	SMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

B.3.1.1.6. *Garabrant et al. (1988).*

B.3.1.1.6.1. ***Author's abstract.***

> A retrospective cohort mortality study was conducted among men and women employed for four or more years, between 1958 and 1982, at an aircraft manufacturing company in San Diego County. Specific causes of death under investigation included cancer of the brain and nervous system, malignant melanoma, and cancer of the testicle, which previous reports have suggested to be associated with work in aircraft manufacturing. Follow-up of the cohort of 14,067 subjects for a mean duration of 15.8 yr from the date of first employment resulted in successful tracing of 95% of the cohort and found 1,804 deaths through 1982. Standardized mortality ratios (SMRs) were calculated based on U. S. national mortality rates and separately based on San Diego County mortality rates. Mortality due to all causes was significantly low (SMR = 75), as was mortality due to all cancer (SMR = 84). There was no significant excess of cancer of the brain, malignant melanoma, cancer of the testicle, any other cancer site, or any other category of death. Additional analyses of cancer sites for which at least ten deaths were found and for which the SMR was at least 110 showed no increase in risk with increasing duration of work or in any specific calendar period. Although this study found no significant excesses in cause-specific mortality, excess risks cannot be ruled out for those diseases that have latency periods in excess of 20 to 30 yr, or for exposures that might be restricted to a small proportion of the cohort.

B.3.1.1.6.2. ***Study description and comment***. This study reported on the overall mortality of a cohort of workers in the aircraft manufacturing industry in southern California who had worked 1 day at the facility and had at least 4 years duration of employment. Fifty-four (54) percent of cohort entered cohort at beginning date (1-1-1958). This is a survivor cohort. This study lacks exposure assessment for study subjects. The only exposure metric was years of work. Examination of jobs held by 70 study subjects, no details provided in paper on subject selection criteria, identified 37% as having possible trichloroethylene TCE exposure, but no information was presented on how they were exposed, frequency or duration of exposure, or job titles associated with exposure. No information is provided on possible trichloroethylene exposure to the remaining ~14,000 subjects in this cohort. The exposure assignment in this study was insufficient to define exposures of the cohort and the frequency of exposures was likely low. Given the enormous misclassification on exposure, the effect of exposure would have to be very large to be detected as an overall risk for the population. Null findings are to be expected due to bias likely associated with a survivor cohort and to exposure misclassification. Therefore, this study provides little information on whether trichloroethylene is related to disease risk.

Garabrant DH, Held J, Langholz B, Bernstein L. 1988. Mortality of Aircraft Manufacturing Workers in Southern California. Am J Ind Med 13:683−693.

Langholz B, Goldstein L. 1996. Risk Set Sampling in Epidemiologic Cohort Studies. Stat Sci 11:35−53.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	"Our objects were to evaluate the oval mortality among the [aircraft manufacturing] workers and to test the hypotheses that brain tumors, malignant melanoma, and testicular neoplasms are associated with work in this industry." [Introduction] This study was not designed to evaluate any specific exposure, but rather employment in aircraft manufacturing industry.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	14,067 males and females working at least 4 yrs with a large aircraft manufacturing company and who had worked for at least 1 day at a factory in San Diego County, CA. Person-year accrued from the anniversary date of an individual's 4th yr of service or from 1-1-1958 to end of follow-up 12-31-1982. External referents—age-, race-, sex-, calendar year- and cause-specific mortality rates of United States population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD revision in effect at the date of death. Lymphomas in 4 groupings: lymphosarcoma and reticulosarcoma, HD, leukemia and aleukemia, and other.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD revision in effect at the date of death. Lymphomas in 4 groupings: lymphosarcoma and reticulosarcoma, HD, leukemia and aleukemia, and other.
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure assessment is lacking for all subjects except 70 deaths (14 esophageal and 56 others) who were included in a nested case-control study. Of the 362 jobs held by these 70 subjects, 37% were identified as having potential for TCE exposure.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	4.7% with unknown vital status.
>50% cohort with full latency	Average 16 yr follow-up.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,804 deaths (12.8% of cohort), 453 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, race, sex, and calendar year.
Statistical methods	SMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	SMR analysis, adequate; Published paper lacks documentation of nested case-control study of esophageal cancer.

B.3.1.2. *Cancer Incidence Studies Using Biological Monitoring Databases*

Finland and Denmark historically have maintained national databases of biological monitoring data obtained from workers in industries where toxic exposures are a concern. Legislation required that employers provide workers exposed to toxic hazards with regular health examinations, which must include biological monitoring to assess the uptake of toxic chemicals, including trichloroethylene. In Sweden, the only local producer of trichloroethylene operated a free exposure-surveillance program for its customers, measuring U-TCA. These programs used the linear relationship found for average inhaled trichloroethylene versus U-TCA: trichloroethylene (mg/m^3) = 1.96; U-TCA (mg/L) = 0.7 for exposures lower than 375 mg/m^3 (69.8 ppm) (Ikeda et al., 1972). This relationship shows considerable variability among individuals, which reflects variation in urinary output and activity of metabolic enzymes. Therefore, the estimated inhalation exposures are only approximate for individuals but can provide reasonable estimates of group exposures. There is evidence of nonlinear formation of U-TCA above about 400 mg/m^3 or 75 ppm of trichloroethylene. The half-life of U-TCA is about 100 hours. Therefore, the U-TCA value represents roughly the weekly average of exposure from all sources, including skin absorption. The Ikeda et al. (1972) relationship can be used to convert urinary values into approximate airborne concentration, which can lead to misclassification if tetrachloroethylene and 1,1,1-trichloroethane are also being used because they also produce U-TCA. In most cases, the Ikeda et al. relationship (1972) provides a rough upper boundary of exposure to trichloroethylene.

B.3.1.2.1. *Hansen et al. (2001).*

B.3.1.2.1.1. ***<u>Author's abstract.</u>***

> Human evidence regarding the carcinogenicity of the animal carcinogen trichloroethylene (TCE) is limited. We evaluated cancer occurrence among 803 Danish workers exposed to TCE, using historical files of individual air and urinary measurements of TCE-exposure. The standardized incidence ratio (SIR) for cancer overall was close to unity for both men and women who were exposed to TCE. Men had significantly elevated SIRs for non-Hodgkin's lymphoma (SIR = 3.5; n = 8) and cancer of the esophagus (SIR = 4.2; n = 6). Among women, the SIR for cervical cancer was significantly increased (SIR = 3.8; n = 4). No clear dose-response relationship appeared for any of these cancers. We found no increased risk for kidney cancer. In summary, we found no overall increase in cancer risk among TCE-exposed workers in Denmark. For those cancer sites where excesses were noted, the small numbers of observed cases and the lack of dose-related effects hinder etiological conclusions.

B.3.1.2.1.2. ***<u>Study description and comment</u>***. This Danish study evaluated cancer incidence in a small cohort of individuals (n = 803) who had been monitored for trichloroethylene exposures in a national surveillance program between 1947 and 1989 for U-TCA or TCE in breath since 1974. In all, 2,397 samples were analyzed for U-TCA of workers at 275 companies and 472 breathing zone samples of TCE from workers at 81 companies. Individual workers could not be identified for roughly one-third of the U-TCA measurements and 50% of breathing zone measurements; many of the individuals most likely had died prior to 1968, the start of the Central Population Registry from which workers were identified and follow-up for cancer incidence. A cohort of 658 males and 145 females were identified from the remaining 1,519 U-TCA and 245 air-TCE measurements. Only two of 803 cohort subjects had both urine and air measurements. Follow-up for cancer incidence ended as of 12-31-1996.

The retirement and measurement records contained general information about the type of employer and the subject's job. The subjects in this study came predominantly from the iron and metal industry with jobs such as metal-product cleaner. Each subject had 1 to 27 measurements of U-TCA measurements, an average of 2.2 per subject, going back to 1947. Using the linear relationship from Ikeda et al. (1972), the historic median exposures estimated from the U-TCA concentrations were low: 9 ppm for 1947 to 1964, 5 ppm for 1965 to 1973, 4 ppm for 1974 to 1979, and 0.7 ppm for 1980 to 1989. However, the distributions were highly skewed. Additionally, 5% of the cohort had urine or air samples below the limit of detection. Overall, median exposure in this cohort was 4 ppm and suggests that, in general, workers in a wide variety of industry and job groups and identified as "exposed" in this study had low TCE intensity exposures. Overall, the cohort in this study is small, drawn from a wide variety of industries, predominantly degreasing and metal cleaning, and had generally low exposures (most less than 20 ppm). The study has a lower power to examine TCE exposure and cancer for these reasons.

Hansen J, Raaschou-Nielsen O, Christensen JM, Johansen I, McLaughlin JK, Lipworth L, Blot WJ, Olsen JH. 2001. Cancer incidence among Danish workers exposed to trichloroethylene. J Occup Environ Med 43:133−139.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From introduction—A study of incidence was carried out to address shortcomings in earlier TCE studies related to the lack of direct exposure information and to assessment of mortality as opposed to incidence.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	803 subjects identified from biological monitoring of urine TCA from 1947−1989 (1,519 measurements) or breathing zone TCE since 1974 (245 measurements) and who were alive as of 1968, followed to 1996. External referents—cancer incidence rates of Danish population (age-, sex-, calendar years-, and site-specific).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD, 7th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Biological marker of TCE in urine or in breath used to assign TCE exposure to cohort subject. Historic median exposures estimated from the U-TCA were low: 9 ppm for 1947 to 1964, 5 ppm for 1965 to 1973, 4 ppm for 1974 to 1979, and 0.7 ppm for 1980 to 1989. **Overall, median TCE exposure to cohort was 4 ppm (arithmetic mean, 12 ppm).**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No.
>50% cohort with full latency	Unable to determine given insufficient information in paper; however, text notes follow-up for most subjects achieved a full latency.

CATEGORY E: INTERVIEW TYPE	
<90% Face-to-Face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	128 incident cancers among 804 cohort subjects (15%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex and calendar year.
Statistical methods	SIR, Life table analysis.
Exposure-response analysis presented in published paper	Yes, as dichotomous variable for mean exposure (<4 ppm, 4+ ppm) and for cumulative exposure.
Documentation of results	Adequate.

SIR = standardized incidence ratio.

B.3.1.2.2. *Anttila et al. (1995).*

B.3.1.2.2.1. ***Author's abstract.***

> Epidemiologic studies and long-term carcinogenicity studies in experimental animals suggest that some halogenated hydrocarbons are carcinogenic. To investigate whether exposure to trichloroethylene, tetrachloroethylene, or 1,1,1-trichloroethane increases carcinogenic risk, a cohort of 2050 male and 1924 female workers monitored for occupational exposure to these agents was followed up for cancer incidence in 1967 to 1992. The overall cancer incidence within the cohort was similar to that of the Finnish population. There was an excess of cancers of the cervix uteri and lymphohematopoietic tissues, however. Excess of pancreatic cancer and non-Hodgkin lymphoma was seen after 10 years from the first personal measurement. Among those exposed to trichloroethylene, the overall cancer incidence was increased for a follow-up period of more than 20 years. There was an excess of cancers of the stomach, liver, prostate, and lymphohematopoietic tissues combined. Workers exposed to 1,1,1-trichloroethane had increased risk of multiple myeloma and cancer of the nervous system. The study provides support to the hypothesis that trichloroethylene and other halogenated hydrocarbons are carcinogenic for the liver and lymphohematopoietic tissues, especially for non-Hodgkin lymphoma. The study also documents excess of cancers of the stomach, pancreas, cervix uteri, prostate, and the nervous system among workers exposed to solvents.

B.3.1.2.2.2. ***Study description and comment***. This Finnish study evaluated cancer risk in a small cohort of individuals (2,050 males and 1,924 females) who had been monitored between 1965 and 1982 for exposures to trichloroethylene by measuring their U-TCA. The main source of exposure was identified as degreasing or cleaning metal surfaces. Some workplaces identified rubber work, gluing, and dry-cleaning. There was an average of 2.7 measurements per person. Using the Ikeda et al. (1972) conversion relationship, the exposure for trichloroethylene was approximately 7 ppm in 1965, which declined to approximately 2 ppm in 1982; the 75th percentiles for these dates were 14 and 7 ppm, respectively. The maximum values for males were approximately 380 ppm during 1965 to 1974 and approximately 96 ppm during 1974 to 1982. Females showed a similar pattern over time but had somewhat higher exposures than males before the 1970s. Median TCE exposure for females of 4 ppm compared to 3 ppm for males; maximum values were similar for both sexes. Duration of exposure was counted from the first measurement of U-TCA, which might underestimate the length of exposure. Without job histories, the length of exposure is uncertain. Another concern is the sampling strategy; it was not reported how the workers were chosen for monitoring. Therefore, it is not clear what biases might be present, especially the possibility of under sampling highly exposed workers.

Overall, this TCE exposed cohort drawn from a wide variety of industries was twice the size of other Nordic biomonitoring studies (Axelson et al., 1994; Hansen et al., 2001) with urine TCA measurements from a more recent period, 1965 to 1982, compared to other Nordic studies of Danish cohorts, 1947 to 1980s, or Swedish cohorts, 1955 to 1975 (Axelson et al., 1994; Hansen et al., 2001; Raaschou-Nielsen et al., 2002). Exposures to trichloroethylene were generally low, less than 14 ppm for the 75th percentile of all measurements, and median TCE exposures decreasing from 7 ppm to 2 ppm over the 17-year period. The medians are similar to estimated exposures to Danish workers with biological markers of U-TCA (Hansen et al., 2001; Raaschou-Nielson et al., 2001). The duration of exposure was uncertain.

Anttila A, Pukkala E, Sallmen M, Hernberg S, Hemminki K. 1995. Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. J Occup Environ Med 37:797−806.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, study aim was to assess cancer incidence among workers biologically monitored for exposure to TCE, PERC, and 1,1,1-trichloroethane.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	3, 976 subjects identified from biological monitoring of urine TCA between 1965 to 1982; PERC in blood, 1974 to 1983; and, 1,1,1-trichloroethane in blood, 1975 to 1983 (a total of 10.743 measurements). 109 of cohort subjects with TCE poisoning report between 1965 to 1976. Follow-up for mortality between 1965 to 1991 and for cancer between 1967 to 1992. TCE subcohort—3,089 (1,698 males, 1,391 females). External referents—age-, sex-, calendar year-, and site-specific cancer incidence rates of the Finnish population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality and cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD, 7th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Biological marker of TCE in urine used to assign TCE exposure for TCE subcohort. There were on average 2.5 U-TCA measurements per individual. 6% of cohort had measurements for 2 or all three solvents. The overall median of U-TCA for females was 8.3 mg/L and 6.3 mg/L for males, and before 1970, 10 to 13 mg/L for females and 13 to 15 mg/L for males. **Using Ikeda et al. (1972) relationship for U-TCA and TCE concentration, median TCE exposures over the period of study were roughly <4−9 ppm (median, 4 ppm; arithmetic mean, 6 ppm).**

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No.
>50% cohort with full latency	Yes, 18 yr mean follow-up period.
CATEGORY E: INTERVIEW TYPE	
<90% Face-to-Face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	208 cancers among 3,089 TCE-exposed subjects (7%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and calendar year.
Statistical methods	SMR and SIR, Life table analysis.
Exposure-response analysis presented in published paper	Yes, U-TCA as dichotomous variable (<6 ppm, 6+ ppm).
Documentation of results	Adequate for SIR analysis; details on SMR analysis of TCE subcohort are few.

PERC = perchloroethylene, SIR = standardized incidence ratio.

B.3.1.2.3. *Axelson et al. (1994).*

B.3.1.2.3.1. ***Author's abstract.***

> There is limited evidence for mutagenicity and carcinogenicity of trichloroethylene (TRI) in experimental test systems. Whether TRI is a human carcinogen is unclear, however. This paper presents an update and extension of a previously reported cohort of workers exposed to TRI, in total 1670 persons. Among men (n = 1421), the overall standardized mortality ratio (SMR) and cancer morbidity ratio (SIR) were close to the expected, with SMR, 0.97; 95% confidence interval (CI), 0.86 to 1.10; and SIR, 0.96; 95% CI, 0.80 to 1.16, respectively. The cancer mortality was significantly lower than expected (SMR, 0.65; 95% CI, 0.47 to 0.89), whereas an increased mortality from circulatory disorders (cardiovascular, cerebrovascular) was of borderline significance (SMR, 1.17; 95% CI, 1.00 to 1.37). No significant increase of cancer of any specific site was observed, except for a doubled incidence of nonmelanocytic skin cancer without correlation with the exposure categories. In the small female subcohort (n = 249), a nonsignificant increase of cancer and circulatory deaths was observed (SMR, 1.53 and 2.02, respectively). For both genders, however, excess risks were largely confined to groups of workers with lower exposure levels or short duration of exposure or both. It is concluded that this study provides no evidence that TRI is a human carcinogen, i.e., when the exposure is as low as for this study population.

B.3.1.2.3.2. ***Study description and comment***. This Swedish study evaluated cancer risk in a small cohort of individuals (1,421 males and 249 females), who were monitored for U-TCA as part of a surveillance system by the trichloroethylene producer during 1955 to 1975. Both mortality between 1955 and 1986 and cancer morbidity between 1958 and 1987 are assessed in males only due to the small number of female subjects. Eighty-one percent of the male subjects had low exposures (<50 mg/L), corresponding to an airborne concentration of trichloroethylene of approximately 20 ppm. There was uncertainty about the beginning and end of exposure. Exposure was assumed to begin with the first urine sample and to end in 1979 (the reason for this date is unclear). Because the investigators did not have job histories, there is considerable uncertainty about the duration of exposure. No information is, additionally, presented to evaluate if a large proportion of the cohort had a full latency period for cancer development. Most subjects appear to have had short durations of exposure, but these might have been underestimated. Another concern is the sampling strategy. It was not reported how the workers were chosen for monitoring. Therefore, it is not clear what biases could be present in the data, especially the possibility of under sampling highly exposed workers.

Overall, this study had a small cohort drawn from a wide variety of industries, predominantly from industries involving degreasing and metal cleaning. Exposure to trichloroethylene was generally low (most less than 20 ppm). The duration of exposure was uncertain and bias related to under sampling of higher exposed workers is possible but can not be evaluated.

Axelson O, Selden A, Andersson K, Hogstedt C. 1994. Updated and expanded Swedish cohort study on trichloroethylene and cancer risk. J Occup Environ 36:556−562.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes- "This paper present an update and extension of a previously reported cohort of workers exposure to TCE."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,670 subjects (1,421 males, 249 females) with records of biological monitoring of urine TCA from 1955 and 1975. Analysis restricted to 1,421 males. External referents—age-, sex-, calendar year-, site-specific mortality or cancer incidence rates of Swedish population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence from 1958 to 1987 and all-cause mortality from 1955 to 1986.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD, 7th revision. ICD, 8th revision from 1975 onward for all lympho-hematopoietic system cancers.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Biological marker of TCE in urine used to assign TCE exposure to cohort subject. No extrapolation of U-TCA data to air-TCE concentration. **Roughly ¾ of cohort had U-TCA concentrations equivalent to <20 ppm TCE.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No
>50% cohort with full latency	Insufficient to estimate for full cohort; however, 42% of person years in subjects with 2+ exposure years also had 10+ yrs of latency.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	229 deaths (16% of male subjects). 107 incident cancer cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and calendar year.
Statistical methods	SMR—age, sex, and calendar-year. SIR—analyses restricted to males—age and calendar-year.
Exposure-response analysis presented in published paper	Yes, by 3 categories of U-TCA concentration.
Documentation of results	Adequate.

SIR = standardized incidence ratio.

B.3.1.3. *Studies in the Taoyuan Region of Taiwan*

B.3.1.3.1. *Sung et al. (2008, 2007).*

B.3.1.3.1.1. ***Sung et al. (2008) abstract.***

> There is limited evidence on the hypothesis that maternal occupational exposure near conception increases the risk of cancer in offspring. This study is to investigate whether women employed in an electronics factory increases childhood cancer among first live born singletons. We linked the databases of Birth Registration and Labor Insurance, and National Cancer Registry, which identified 40,647 female workers ever employed in this factory who gave 40,647 first live born singletons, and 47 of them developed cancers during 1979-2001. Mothers employed in this factory during their periconceptional periods (3 months before and after conception) were considered as exposed and compared with those not employed during the same periods. Poisson regression model was constructed to adjust for potential confounding by maternal age, education, sex, and year of birth. Based on 11 exposed cases, the rate ratio of all malignant neoplasms was increased to 2.26 [95% confidence interval (CI), 1.12-4.54] among children whose mothers worked in this factory during periconceptional periods. The RRs were associated with 6 years or less (RR=3.05; 95% CI, 1.20-7.74) and 7-9 years (RR=2.49; 95% CI, 1.26-4.94) of education compared with 10 years or more. An increased association was also found between childhood leukemia and exposed pregnancies (RR=3.83; 95% CI, 1.17-12.55). Our study suggests that maternal occupation with potential exposure to organic solvents during periconception might increase risks of childhood cancers, especially for leukemia.

B.3.1.3.1.2. ***Sung et al. (2007) abstract.***

> **Background** In 1994, a hazardous waste site, polluted by the dumping of solvents from a former electronics factory, was discovered in Taoyuan, Taiwan. This subsequently emerged as a serious case of contamination through chlorinated hydrocarbons with suspected occupational cancer. The objective of this study was to determine if there was any increased risk of breast cancer among female workers in a 23-year follow-up period. **Methods** A total of 63,982 female workers were retrospectively recruited from the database of the Bureau of Labor Insurance (BLI) covering the period 1973-1997; the data were then linked with data, up to 2001, from the National Cancer Registry at the Taiwanese Department of Health, from which standardized incidence ratios (SIRs) for different types of cancer were calculated as compared to the general population. **Results** There were a total of 286 cases of breast cancer, and after adjustment for calendar year and age, the SIR was close to 1. When stratified by the year 1974 (the year in which the regulations on solvent use were promulgated), the SIR of the cohort of workers who were first employed prior to 1974 increased to 1.38 (95% confidence interval, 1.11-1.70). No such trend was discernible for workers employed after 1974. When 10 years of employment was considered, there was a

further increase in the SIR for breast cancer, to 1.62. Those workers with breast cancer who were first employed prior to 1974 were employed at a younger age and for a longer period. Previous qualitative studies of interviews with the workers, corroborated by inspection records, showed a short-term high exposure to chlorinated alkanes and alkenes, particularly trichloroethylene before 1974. There were no similar findings on other types of cancer. **Conclusions** Female workers with exposure to trichloroethylene and/or mixture of solvents, first employed prior to 1974, may have an excess risk of breast cancer.

B.3.1.3.1.3. ***Study description and comment***. Sung et al. (2007) examine breast cancer incidence among females in a cohort of electronic workers with employment at one factory in Taoyuan, Taiwan between 1973 and 1992, date of factory closure and followed to 2001. Some female subjects in Sung et al. (2007) overlap those in Chang et al. (2003, 2005) who included workers from the same factory whose employment dates were between 1978 and 1997, the closing date of the study a date of vital status ascertainment. A total of 64,000 females were identified with 63,982 in the analysis after the exclusion of 15 women with less than one full day of employment and three women with cancer diagnoses prior to the time of first employment; approximately 6,000 fewer female subjects compared to Chang et al. (2005) (70,735 females). Cancer incidence between 1979 and 2001 as identified using the National Cancer Registry which contained 80% of all cancer cases in Taiwan (Parkin et al., 2002) is examined using life table methods with exposure lag periods of 5−15 years, depending on the cancer site, and cancer rates from the larger Taiwanese population as referent.

Company employment records were lacking and the cohort was constructed using the Bureau of Labor Insurance database that contained computer records since 1978 and paper records for the period 1973 to 1978. Duration of employment was calculated from the beginning of coverage of labor insurance and is likely an underestimate. Labor insurance hospitalization data and a United Labor Association list of names were used to verify cohort completeness. While these sources may have been sufficient to identified current employees, their ability to identify former employees may be limited, particularly from the hospitalization data if the subject's current employer was listed.

This study assumes all employees in the factory were exposed to chlorinated organic solvent vapors and the primary exposure index was duration of employment at the plant. Most subjects had employment durations of <1 year (65%). Durations of exposure were likely underestimated as dates of commencement and termination of insurance coverage were incomplete, 7.5% and 6%, respectively. There is little to no information on chemical usage and exposure assignment to individual cohort subjects. As reported in Chang et al. (2003, 2005), records of the Department of Labor Inspection ad Bureau of International Trade, in addition, to recall of former industrial hygienists were used to identify chemicals used after 1975 in the plants. No information is available prior to this date.

Sung et al. (2008) presents an analysis of childhood cancer incidence (1979−2001) among first liveborn singleton births (1978 and 2001) of female subjects employed at the plant during a period 3 months before and after beginning of pregnancy, an estimate derived by Sung et al. (2008) from the date of birth and estimated length of gestation plus 14 days. Sung et al. (2007) used Poisson regression methods and cancer incidence among first liveborn births of all other women in Taiwan in the same time to calculate relative risks associated with leukemia risk among exposed offspring. Poisson models were adjusted for maternal age, maternal educational level, child's sex, and year of birth. A total of 8,506 first born singleton births among 63,982 female subjects were identified from the Taiwan Birth Registry database, and 11 cancers, including 6 leukemia cases and no brain/central nervous system (CNS) cases identified from the National Cancer Registry database.

Overall, these studies do not provide substantial weight for determining whether trichloroethylene may cause increased risk of disease. The lack of TCE-assessment to individual cohort subjects; grouping cohort subjects with different exposure potential, both to different solvents and different intensities; and deficiencies in the record system used to construct the cohort introduce uncertainty.

Sung T-I, Chen P_C, Lee L J-H, Lin Y-P, Hsieh G-Y, Wang J-D. 2007. Increased standardized incidence ratio of breast cancer in female electronics workers. BMC Public Health 7:102. http://www.biomedcentral.com/content/pdf/1471-2458-7-102.pdf.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "This study is to investigate whether women employed in an electronics factory increases childhood cancer among first live born singletons." This study was not able to evaluate TCE exposures uniquely.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	63,982 females, some who were also subjects were also in cohort of Chang et al. (2003, 2005) with 70,735 females. Cohort initially established using labor insurance records (computer records after 1978 and paper records from 1973 and 1978) in the absence of company records. Cohort definition dates are not clearly identified. Cohort identified from records covering period 1973 and 1997 with vital status ascertained as of 2001. Factory closed in 1992. External referents: age-, calendar-, and sex-specific incidence rates of the Taiwanese general population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence as ascertained from National (Taiwan) Cancer Registry (80% of all cancers reported to Registry).
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-Oncology, a supplement to ICD-9.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All employees assumed to be potentially exposed to chlorinated organic solvent vapors; study does not assign potential chemical exposures to individual subjects. No information on specific chemical exposures or intensity. Limited identification of solvents used in manufacturing process from the period after 1975 inferred from records of Department of Labor Inspection, Bureau of International Trade, and former industrial hygienists recall. No information on solvent usage was available before 1975. Exposure index defined as duration of exposure which was likely underestimated. 21% of cohort with ≥10 yrs duration of employment and 53% with <1 yr duration.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information on loss to follow-up. Subject was assumed disease free at end of follow-up if lacking cancer diagnosis as recorded in the National Cancer Registry.
>50% cohort with full latency	No, 57% of cohort employed after November 21, 1978.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,311 cancer cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age-, calendar-, and sex-specific incidence rates.
Statistical methods	SIR, analyses include a lag period of 5, 10, or 15 yrs since first employment (as indicated by labor insurance record).

Exposure-response analysis presented in published paper	Cancer incidence examined by duration of employment; however, employment durations were likely underestimates as dates of commencement and termination dates on of insurance coverage date were incomplete and misclassification bias is likely present.
Documentation of results	Inadequate—analyses that do not include a lag are not presented nor discussed in published paper or in supplemental documentation.

SIR = standardized incidence ratio.

Sung T-I, Wang J-D, Chen P_C. 2008. Increased risk of cancer in the offspring of female electronics workers. Reprod Toxicol 25:115−119.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract “The study was designed to examine whether breast cancer risk in females was increased, as had been observed in Chang et al. (2003, 2005) in a cohort with earlier employment dates.” This study was not able to evaluate TCE exposure.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	11 cancers among 8,506 first born singleton births between 1978−2001 in 63,982 female subjects of Sung et al. (2007). Cancers identified from National Cancer Registry and births identified from Taiwan Birth Registration database. External referents: cancer incidence among all other first birth singleton births among Taiwanese females over the same time period.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence as ascertained from National (Taiwan) Cancer Registry (80% of all cancers reported to Registry).
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin’s lymphoma	ICD-Oncology, a supplement to ICD-9, specific leukemia subtypes not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All births were among subjects with employment at factory during a period 3 mos before and after beginning of pregnancy. All mothers were assumed potentially exposed to chlorinated organic solvent vapors; specific solvents are not identified nor assigned to individual subjects. Limited identification of solvents used in manufacturing process from the period after 1975 inferred from records of Department of Labor Inspection, Bureau of International Trade, and former industrial hygienists recall. No information on solvent usage was available before 1975.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information on loss to follow-up for females in Sung et al. (2007).

>50% cohort with full latency	66% of births would have been 16 yrs of age as of 2001, the date cancer incidence ascertainment ended.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	11 cancer cases among 8,506 first born singleton births.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Maternal age, maternal educational level, child's sex and child's year of birth.
Statistical methods	Poisson regression using childhood cancer incidence among all other first live born children in Taiwan during same time period.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.1.3.2. *Chang et al. (2005, 2003).*

B.3.1.3.2.1. ***Chang et al. (2005) abstract.***

A retrospective cohort morbidity study based on standardized incidence ratios (SIRs) was conducted to investigate the possible association between exposure to chlorinated organic solvents and various types of cancers in an electronic factory. The cohort of the exposure group was retrieved from the Bureau of Labor Insurance (BLI) computer database records dating for 1978 through December 31, 1997. Person-year accumulation began on the date of entry to the cohort, or January 1, 1979 (whichever came later), and ended on the closing date of the study (December 31, 1997), if alive with out contracting any type of cancers, or the date of death, or the date of the cancer diagnosis. Vital status and cases of cancer of study subjects were determined from January 1, 1979 to December 31, 1997 by linking cohort data with the National Cancer Registry Database. The cancer incidence of the general population was used fro comparison. After adjustment for age and calendar year, only SIR for breast cancer in the exposed female employees were significantly elevated when compared with the Taiwanese general population, based on the entire cohort without exclusion. The SIR of female breast cancer also showed a significant trend of period effect, but no significant dos-response relationship on duration of employment. Although the total cancer as well as the cancer for the trachea, bronchus[,] and lung for the entire female cohort was not significantly elevated, trend analysis by calendar-year interval suggested an upward trend. However, when duration of employment or latency was taken into consideration, no significantly elevated SIR was found for any type of cancer in either male or female exposed workers. In particular, the risk of female breast cancer was not indicated to be increased. No significant dose-response relationship on duration of employment and secular trend was found for the above-mentioned cancers. This study provides no evidence that exposure to chlorinated organic solvents at the electronics factory was associated with elevated human cancers.

B.3.1.3.2.2. ***Chang et al. (2003) abstract.***

PURPOSE: A retrospective cohort mortality study based on standardized mortality ratios (SMRs) was conducted to investigate the possible association between exposure to chlorinated organic solvents and various types of cancer deaths. **METHODS**: Vital status and causes of death of study subjects were determined from January 1, 1985 to December 31, 1997, by linking cohort data with the National Mortality Database. Person-year accumulation began on the date of entry to the cohort, or January 1, 1985 (whichever came later), and ended on the closing date of the study (December 31, 1997), if alive; or the date of death. **RESULTS**: This retrospective cohort study examined cancer mortality among 86,868 workers at an electronics factory in the northern Taiwan. Using various durations of employment and latency and adjusting for age and calendar year, no significantly elevated SMR was found for any cancer in either male or female exposed workers when compared with the general Taiwanese population. In particular, the risk of female breast cancer was not found to be increased. Although ovarian cancer suggested an upward trend when analyzed by length of employment, ovarian cancer risk for the entire female cohort was not elevated. **CONCLUSIONS**: It is concluded that this study provided no evidence that exposure to chlorinated organic solvents was associated with human cancer risk.

B.3.1.3.2.3. ***Study description and comment***. Both Chang et al. (2003) and Chang et al. (2005) studied a cohort of 86,868 subjects employed at an electronics factory between 1985 and 1997, and both administrative and nonadministrative (blue-collar) workers were included in the cohort. Cancer incidence between 1979 and 1997 was presented by Chang et al. (2005) and cancer mortality from 1985 to 1997 in Chang et al. (2003). The cohort was predominately composed of females. The factory operated between 1968 and 1992, and the inclusion in the cohort of subjects after factory closure is questionable. Incidence was ascertained from the Taiwan National Cancer Registry which contains 80% of all cancer cases in Taiwan (Parkin et al., 2002). The factory could be divided into three plants by manufacturing process: manufacture of television remote controls, manufacture of solid state and integrated circuit products, and manufacture of printed circuit boards. Furthermore, a factory waste disposal site was found to have contaminated the underground water supply of area communities with organic solvents, however, Chang et al. (2005) does not provide information on possible exposure to factory employees through ingestion. The analysis of communities adjacent to the factory is described in Lee et al. (2003).

Company employment records were lacking and the cohort was constructed using the Bureau of Labor Insurance database that contained computer records since 1978. Labor insurance hospitalization data and a United Labor Association list of names were used to verify cohort completeness. While these sources may have been sufficient to identified current employees, their ability to identify former employees may be limited, particularly from the hospitalization data if the subject's currently employer was listed.

All employees in the factory were assumed with potential exposure to chlorinated organic solvent vapors with duration of employment at the factory as the exposure surrogate. Subjects had varying exposure potentials and employment durations of <1 year (65% of cohort in Chang et al., 2005). Durations of exposure were likely underestimated as dates of commencement and termination of insurance coverage were incomplete, 7.5 and 6%, respectively. Three plants comprised the factory and with different production processes. A wide variety of organic solvents were used in each process including dichloromethane, toluene, and methyl ethyl alcohol, used at all three plants, and perchloroethylene, propanol, and dichloroethylene which was used at one of the 3 plants Chang et al. (2005). Records of the Department of Labor Inspection and Bureau of International Trade, in addition, to recall of former industrial hygienists were used to identify chemicals used after 1975 in the plants. No information is available prior to this date. These sources documented the lack of TCE use between 1975 and 1991 and perchloroethylene was after 1981. No information was available on TCE and perchloroethylene usage during other periods. Given the period of documented lack of TCE usage is before the cohort start date of 1978 and factory closure, there is great uncertainty of TCE exposure to cohort subjects.

Overall, both studies are not useful for determining whether trichloroethylene may cause increased risk of disease. The lack of TCE-assessment to individual cohort subjects and uncertainty of TCE usage in the factory; potential bias likely introduced through missing employment dates; and, examination of incidence using broad organ-level categories, i.e., lymphatic and hematopoietic tissue cancer together, decrease the sensitivity of this study for examining trichloroethylene and cancer. Furthermore, few cancers are expected, 1% of the cohort expected with cancer, and results in low statistical power from the cohort's young average age of 39 years.

Chang Y-M, Tai C-F, Yang S-C, Lin R, Sung F-C, Shin T-S, Liou S-H. 2005. Cancer Incidence among Workers Potentially Exposed to Chlorinated Solvents in An Electronics Factory. J Occup Health 47:171−180.

Chang Y-M, Tai C-F, Yang S-C, Chan C-J, S Shin T-S, Lin RS, Liou S-H. 2003. A cohort mortality study of workers exposed to chlorinated organic solvents in Taiwan. Ann Epidemiol 13:652−660.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The study was not designed to uniquely evaluate TCE exposure but rather chlorinated solvents exposures. From abstract: "... to investigate the possible association between chlorinated organic solvents and various types of cancer in an electronics factory." This study is quite limited to meet stated hypothesis by the inclusion of all factory employees in the cohort and lack of exposure assessment on individual study subjects to TCE, specifically, and to chlorinated solvents, generally.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	*n* = 86,868 in cohort. Cohort initially established using labor insurance records in the absence of company records. Cohort definition dates are not clearly identified. Cohort identified from labor insurance records covering period 1978 and 1997; yet, plant closed in 1992. All subjects followed through 1997. Paper states cohort was completely identified; however, former workers who were eligible for cohort membership may not have been identified if validation sources did not identify former employer. Duration of employment reconstructed from insurance records: ~40% of subjects had employment durations <3 mos, 9% employed >5 yrs, 0.7% employed >10 yrs. External referents: Age-, calendar-, and sex-specific incidence rates of the Taiwanese general population.

CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence as ascertained from National (Taiwan) Cancer Registry (80% of all cancers reported to Registry) (Chang et al., 2005). Mortality. ICD revision is not identified other than that used in 1981 (Chang et al., 2003).
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-Oncology, a supplement to ICD-9 (Chang et al., 2005). ICD, 9th revision was in effect in 1981, but paper does not identify to which ICD revision used to assign cause of death (Chang et al., 2003).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All employees assumed to be potentially exposed to chlorinated organic solvent vapors. No information on specific chemical exposures or intensity. Limited identification of solvents used in manufacturing process from the period after 1975 inferred from records of Department of Labor Inspection, Bureau of International Trade, and former industrial hygienists recall. No information on solvent usage was available before 1975.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information on loss to follow-up. Subject was assumed disease free at end of follow-up if lacking cancer diagnosis as recorded in the National Cancer Registry.
>50% cohort with full latency	Average 16-yr follow-up (incidence) and 12 yrs (mortality).
Other	Subject's age determined by subtracting year of birth from 1997; however, insurance records did not contain DOB for 6% of subjects. Furthermore, commencement and termination dates were incomplete on insurance records, 7 and 6%, respectively.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,031 cancer cases. 1,357 total deaths (1.6% of cohort), 316 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age-, calendar-, and sex-specific incidence rates (Chang et al., 2005) or age-, calendar-, and sex-specific mortality rates (Chang et al., 2003).
Statistical methods	SIR (Chang et al., 2005) and SMR (Chang et al., 2003).
Exposure-response analysis presented in published paper	Cancer incidence and mortality examined by duration of employment; however, employment durations were likely underestimates as dates of commencement and termination dates on of insurance coverage date were incomplete and calculated from date on insurance records. Misclassification bias is likely present.
Documentation of results	Adequate.

SIR = standardized incidence ratio.

B.3.1.4. *Studies of Other Cohorts*

B.3.1.4.1. *Clapp and Hoffman (2008).*

B.3.1.4.1.1. ***Author's abstract.***

> BACKGROUND: In response to concerns expressed by workers at a public meeting, we analyzed the mortality experience of workers who were employed at the IBM plant in Endicott, New York and died between 1969 - 2001. An epidemiologic feasibility assessment indicated potential worker exposure to several known and suspected carcinogens at this plant. METHODS: We used the mortality and work history files produced under a court order and used in a previous mortality analysis. Using publicly available data for the state of New York as a standard of comparison, we conducted proportional cancer mortality (PCMR) analysis. RESULTS: The results showed significantly increased mortality due to melanoma (PCMR = 367; 95% CI: 119, 856) and lymphoma (PCMR = 220; 95% CI: 101, 419) in males and modestly increased mortality due to kidney cancer (PCMR = 165; 95% CI: 45, 421) and brain cancer (PCMR = 190; 95% CI: 52, 485) in males and breast cancer (PCMR = 126; 95% CI: 34, 321) in females. CONCLUSION: These results are similar to results from a previous IBM mortality study and support the need for a full cohort mortality analysis such as the one being planned by the National Institute for Occupational Safety and Health.

B.3.1.4.1.2. ***Study description and comment***. This proportional cancer mortality ratio study of deaths between 1969 and 2001 among employees at an IBM facility in Endicott, NY, who were included on the IBM Corporate Mortality File compared the observed number of site-specific cancer deaths are compared to the expected proportion, adjusted for age, using 10-year rather than 5-year grouping, and sex, of site-specific cancer deaths among New York residents during 1979 to 1998. Of the 360 deaths identified of Endicott employees, 115 deaths were due to cancer, 11 of these with unidentified site of cancer. Resultant proportional mortality ratios estimates do not appear adjusted for race nor does the paper identify whether referent rates excluded deaths among New York City residents or are for New York deaths. The IBM Corporate Mortality File contained names of employees who had worker >5 years, who were actively employed or receiving retirement or disability benefits at time of death, or whose family had filed a claim with IBM for death benefits and Endicott plant employees were identified using worker employment data from the IBM Corporate Employee Resource Information System. Study investigators had previously obtained the IBM Corporate Mortality file through a court order and litigation.

The Endicott plant began operations in 1991 and manufactured a variety of products including calculating machines, typewriters, guns, printers, automated machines, and chip packaging. The most recent activities were the production of printed circuit boards. It was estimated from a National Institute of Occupational Safety and Health (NIOSH) feasibility study that a larger percentage of the plant's employee were potentially exposure to multiple chemicals, including asbestos, benzene, cadmium, nickel compounds, vinyl chloride, tetrachloroethylene, TCE , PCBs, and o-toluidine. Chlorinated solvents were used at the plant until the 1980s. The study does not assign exposure potential to individual study subjects.

This study provides little information on cancer risk and TCE exposure given its lack of worker exposure history information and absence of exposure assignment to individual subjects. Other limitations in this study which reduces interpretation of the observations included incomplete identification of deaths, the analysis limited to only vested employees or to those receiving company death benefits, incomplete identification of all employees at the plant, the inherent limitation of the PMR method and instability of the effect measure particularly in light of bias resulting of excesses or deficits in deaths, and observed differences in demographic (race) between subjects and the referent (New York) population.

Clapp RW, Hoffman K. 2008. Cancer mortality in IBM Endicott plant workers, 1969−2001: an update on a NY production plant. Environ health 7:13.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "…In response to concerns expressed by workers at a public meeting, we analyzed the mortality experience of workers who were employed at the IBM plant in Endicott, New York and died between 1969-2001."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Deaths among IBM workers identified in IBM Corporate Mortality File; workers with ≥5 yrs employment, who were actively employed or receiving retirement or disability benefits at time of death, or whose family had filed a claim with IBM for death benefits. Expected number of site-specific cancer deaths calculated from proportion of cancer deaths among New York residents. Paper does not identify if referent included all New York residents or those living upstate.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD 9.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This study lacks exposure information. TCE and other chemicals were used at the factory and inclusion on the employee list served as a surrogate for TCE exposure of unspecified intensity and duration.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Not able to evaluate given inability to identify complete cohort.
>50% cohort with full latency	Not able to evaluate given lack of work history records.
Other	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	

Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	360 deaths, 115 due to cancer, between 1969−2001.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and gender. No information was available on race and PMRs are unadjusted for race.
Statistical methods	Proportionate mortality ratio.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.1.4.2. *Agency for Toxic Substances and Disease Registry (ATSDR, 2004).*

B.3.1.4.2.1. ***Author's abstract.***

The View-Master stereoscopic slide viewer has been a popular children's toy since the 1950s. For nearly half a century, the sole U.S. manufacturing site for the View-Master product was a factory located on Hall Boulevard in Beaverton, Oregon. Throughout this period, an on-site supply well provided water for industrial purposes and for human consumption. In March 1998, chemical analysis of the View-Master factory supply well revealed the presence of the degreasing solvent trichloroethylene (TCE) at concentrations as high as 1,670 micrograms per liter (µg/L)—the U.S. Environmental Protection Agency maximum contaminant level is 5 µg/L. Soon after the contamination was discovered, the View-Master supply well was shut down. Up to 25,000 people worked at the plant and may have been exposed to the TCE contamination. In September of 2001, the Oregon Department of Human Services (ODHS) entered into a cooperative agreement with the Agency for Toxic Substances and Disease Registry (ATSDR) to determine both the need for and the feasibility of an epidemiological study of the View-Master site. In this report, ODHS compiles the findings of the feasibility investigation of worker exposure to TCE at the View-Master factory.

On the basis of the levels of TCE found in the supply well, the past use of the well as a source of drinking water, and the potential for adverse health effects resulting from past exposure to TCE, ODHS determined that the site posed a public health hazard to people who worked at or visited the plant prior to the discovery of the contamination. Because the use of the View-Master supply well was discontinued when the contamination was discovered in March 1998, the View-Master supply well does not pose a current public health hazard. No other drinking water wells tap into the contaminated aquifer, and the long-term remediation efforts appear to be containing the contamination.

ATSDR and ODHS obtained a list of 13,700 former plant workers from the Mattel Corporation. In collaboration with ATSDR, ODHS conducted a preliminary analysis of mortality and identified excesses in the proportions of deaths due to kidney cancer and pancreatic cancer among the factory's former employees. Although this analysis was limited by the lack of information about the entire worker population and individual exposures to TCE, the preliminary findings underscore the need to fully investigate the impact of TCE exposure on the population of former View-Master workers.

The findings of this feasibility investigation are:

- TCE appears to have been the primary contaminant of the drinking water at the plant;
- Contamination was likely present for a long period of time (estimated to have been present in the groundwater since the mid-1960s);
- A large number were likely exposed to the contamination:
- The primary route of exposure (for the last 18 years the factory operated) was through contaminated drinking water;

- Levels of TCE contamination were 300 time the maximum contaminant levels; and
- A significant portion of the former workers of their next of kin can indeed be located and invited to participate in a public health evaluation of their exposures.

Therefore, ODHS recommends further investigation to include the following:

1. A fate and transport assessment to better establish when TCE reached the supply well, and to provide a historical understanding of the concentration of TCE in the well, and
2. Epidemiological studies among former workers to determine their exposure and whether they have experienced adverse health and reproductive outcomes associated with TCE exposure at the plant, to determine the mortality experience of the population, and to document the cancer incidence in this population.

B.3.1.4.2.2. ***Study description and comment***. This proportionate mortality ratio study of deaths between 1995−2001 among 13,697 former employees at a View-Master toy factory in Beaverton, Oregon contains no exposure information on individual study subjects. The PMR analysis was conducted as a feasibility study for further epidemiologic investigations of these subjects by Oregon Department of Health on behalf of ATSDR, and findings have not been published in the peer-reviewed literature. A former plant owner provided a listing of former employees; however, employees were not identified using IRS records and the roster was known to be incomplete. Additionally, work history records were not available and not information was available on employment length or job title. The goal of the feasibility analysis was to evaluate ability to identify completeness of death identification using several sources.

Monitoring of a water supply well in March 1998 showed detectable concentrations of TCE, and this study assumes all subjects had exposure to TCE in drinking water. TCE had been used in large quantities for metal degreasing at the factory between 1952 and 1980; this activity mostly occurred in the paint shop located in one building. At the time metal degreasing ceased, company records suggested historical use of TCE was up to 200 gallons per month. Historical practices resulted in releases of hazardous substances at the factory site and former employees reported waste TCE from the degreased was transported to other sites on the premises, and discharged to the ground (ATSDR, 2004). Additionally, chemical spills allegedly occurred in the paint shop and one report in 1964 of an inspection of the degreaser indicated atmospheric TCE concentrations above occupational limits. TCE was detected at concentrations between 1,220−1,670 μg/L in four water samples and the Oregon Department of Environmental Quality estimated the well had been contaminated for over 20 years. Other volatile organic compounds (VOCs) besides TCE detected in the supply well water in March 1998 included cis-1,2-dichloroethylene at levels up to 33 μg/L and perchloroethylene at concentrations up to

56-μg/L. The 160-foot-deep supply well was on the property since original construction in 1950 and it supplied water for drinking, sanitation, fire fighting, and industrial use. Connection to municipal water supply occurred in 1956; however, although municipal water was directed to some parts of the plant, the supply well continued to serve the facility's needs, including most of the drinking and sanitary water (ATSDR, 2003).

This study provides little information on cancer risk and TCE exposure given the absence of monitoring data beyond a single time period, absence of estimated TCE concentrations in drinking water, and exposure pathways other than ingestion. Other limitation in this study which reduces interpretation of the observations included incomplete identification of employees with the result of missing deaths likely, the inherent limitation of the PMR method and instability of the effect measure particularly in light of bias resulting of excesses or deficits in deaths, and observed differences in demographic (age and male/female ratio) between subjects and the referent (Oregon) population.

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Feasibility investigation of worker exposure to trichloroethylene at the View-Master Factory in Beaverton, Oregon. Final Report. Submitted by Environmental and Occupational Epidemiology, Oregon Department of Human Services. December 2004.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The goal of this feasibility investigation for a cohort epidemiologic study of former employees at a plant manufacturing stereoscopic slide viewers examined the ability to identify former employees and ascertain vital status.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Name of ~13,000 former employee names were provided to ATSDR by the former plant owner. The current list of employees was known to be incomplete. The proportion of site-specific mortality among workers between 1989−2001 was compared to the proportion expected using all death in Oregon for a similar time period.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD 9 and ICD 10.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This study lacks actual exposure information; work history records were not available. TCE was used at the factory and inclusion on the employee list served as a surrogate for TCE exposure of unspecified intensity and duration.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Not able to evaluate given inability to identify complete cohort.
>50% cohort with full latency	Not able to evaluate given lack of work history records.
Other	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	

Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	616 deaths between 1989−2001.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and gender. No information was available on race and PMRs are unadjusted for race.
Statistical methods	Proportionate mortality ratio.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.1.4.3. *Raaschou-Nielsen et al. (2003).*

B.3.1.4.3.1. ***Author's abstract.***

> Trichloroethylene is an animal carcinogen with limited evidence of carcinogenicity in humans. Cancer incidence between 1968 and 1997 was evaluated in a cohort of 40,049 blue-collar workers in 347 Danish companies with documented trichloroethylene use. Standardized incidence ratios for total cancer were 1.1 (95% confidence interval (CI): 1.04, 1.12) in men and 1.2 (95% CI: 1.14, 1.33) in women. For non-Hodgkin's lymphoma and renal cell carcinoma, the overall standardized incidence ratios were 1.2 (95% CI: 1.0, 1.5) and 1.2 (95% CI: 0.9, 1.5), respectively; standardized incidence ratios increased with duration of employment, and elevated standardized incidence ratios were limited to workers first employed before 1980 for non-Hodgkin's lymphoma and before 1970 for renal cell carcinoma. The standardized incidence ratio for esophageal adenocarcinoma was 1.8 (95% CI: 1.2, 2.7); the standardized incidence ratio was higher in companies with the highest probability of trichloroethylene exposure. In a subcohort of 14,360 presumably highly exposed workers, the standardized incidence ratios for non-Hodgkin's lymphoma, renal cell carcinoma, and esophageal adenocarcinoma were 1.5 (95% CI: 1.2, 2.0), 1.4 (95% CI: 1.0, 1.8), and 1.7 (95% CI: 0.9, 2.9), respectively. The present results and those of previous studies suggest that occupational exposure to trichloroethylene at past higher levels may be associated with elevated risk for non-Hodgkin's lymphoma. Associations between trichloroethylene exposure and other cancers are less consistent.

B.3.1.4.3.2. ***Study description and comment***. Raaschous-Nielsen et al. (2003) examine cancer incidence among a cohort of workers drawn from 347 companies with documented trichloroethylene. Almost half of these companies were in the iron and metal industry. The cohort was identified using the Danish Supplementary Pension Fund, which includes type of industry of a company and a history of employees, for the years 1964 to 1997. Altogether, 152,726 workers were identified of whom 39,074 were white-collar and assumed not to have TCE exposure, 56,970 workers were of unknown status, and 56,578 blue-collar workers, of which 40,049 had been employed at the company for more than 3 months and are the basis of the analysis. The cohort was relatively young, 56% were 38 to 57 years old at end of follow-up, and 29% of subjects were older than 57 years of age. Cancer rates typically increase with increasing ages; thus, the lower age of this cohort likely limits the ability of this study to fully examine TCE and cancer, particularly cancers that may be associated with aging. Observed number of site-specific incident cancers are obtained from 4-1-1968 to the end of 1997 and compared to expected numbers of site-specific cancers based on incidence rates of the Danish population.

A separate exposure assessment was conducted using regulatory agency data from 1947 to 1989 (Raaschou-Nielsen et al., 2002). This assessment identified three factors as increasing potential for TCE exposure, duration of employment, year of first employment, and number of employees, to increase the likelihood of cohort subjects as TCE exposed. The percentage of exposed workers was found to decrease as company size increased: 81% for <50 workers, 51% for 50−100 workers, 19% for 100−200 workers, and 10% for >200 workers. About 40% of the workers in the cohort were exposed (working in a room where trichloroethylene was used). Smaller companies had higher exposures. Median exposures to trichloroethylene were 40−60 ppm for the years before 1970, 10−20 ppm for 1970 to 1979, and approximately 4 ppm for 1980 to 1989. Additionally, an assessment of TCA concentrations in urine of Danish workers suggested a similar trend over time; mean concentrations of 58 mg/L for the period between 1960 and 1964 and 14 mg/L in sample taken between 1980 and 1985 (Raaschou-Nielsen et al., 2001).

Only a small fraction of the cohort was exposed to trichloroethylene. The highest exposures occurred before 1970 at period in which 21.2% of blue-collar workers had begun employment in a TCE-using company. The iron and metal industry doing degreasing and cleaning with trichloroethylene had the highest exposures, with a median concentration of 60 ppm and a range up to about 600 ppm. Overall, strengths of this study include its large numbers of subjects; however, the younger age of the cohort and the small fraction expected with TCE exposure limit the ability of the study to provide information on cancer risk and TCE exposure. For these reasons, positive associations observed in this study are noteworthy.

Raaschou-Nielsen O, Hansen J, McLaughlin JK, Kolstad H, Christensen JM, Tarone RE, Olsen JH. 2003. Cancer risk among workers at Danish companies using trichloroethylene: a cohort study. Am J Epidemiol 158:1182−1192.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was designed to evaluate associations observed in Hansen et al. (2001) with TCE exposure and NHL, esophageal adenocarcinoma, cervical cancer, and liver-biliary tract cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort of 40,049 blue-collar workers employed in 1968 or after with >3 mo employment duration identified by linking 347 companies, who were considered as having a high likelihood for TCE exposure, with the Danish Supplementary Pension Fund to identify employees and with Danish Central Population Registry. External referents are age-, sex-, calendar year-, site-specific cancer incidence rates of the Danish population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence between 4-1-1968 and 12-31-1997 as identified from records of Danish Cancer Registry.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD, 7th revision.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Qualitative exposure assessment. A previous industrial hygiene survey of Danish companies identified several characteristics increase likelihood of TCE exposure-duration of employment, year of 1st employment, and number of employees in company (Raaschou-Nielsen et al., 2002). Exposure index defined as duration of employment. **Median exposures to trichloroethylene were 40−60 ppm for the years before 1970, 10−20 ppm for 1970 to 1979, and approximately 4 ppm for 1980 to 1989. Additionally, an assessment of TCA concentrations in urine of Danish workers suggested a similar trend over time; mean concentrations of 58 mg/L for the period between 1960 and 1964 and 14 mg/L in sample taken between 1980 and 1985 (Raaschou-Nielsen et al., 2001).**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Danish Cancer Registry is considered to have a high degree of reporting and accurate cancer diagnoses.
>50% cohort with full latency	Yes, average follow-up was 18 yrs.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	3.244 cancers (8% of cohort had developed a cancer over the period from 1968 to 1997). Although of a large number of subjects, this cohort is of a young age, 29% of cohort was >57 years of age at end of follow-up.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and calendar year.
Statistical methods	SIR using life-table analysis.

Exposure-response analysis presented in published paper	Yes, duration of employment.
Documentation of results	Adequate.

SIR = standardized incidence ratio.

B.3.1.4.4. *Ritz (1999a).*

B.3.1.4.4.1. *Author's abstract.*

> Data provided by the Comprehensive Epidemiology Data Resource allowed us to study patterns of cancer mortality as experience by 3814 uranium-processing workers employed at the Fernald Feed Materials Production Center in Fernald, Ohio. Using risk-set analyses for cohorts, we estimated the effects of exposure to trichloroethylene, cutting fluids, and kerosene on cancer mortality. Our results suggest that workers who were exposed to trichloroethylene experienced an increase in mortality from cancers of the liver. Cutting-fluid exposure was found to be strongly associated with laryngeal cancers and, furthermore, with brain, hemato- and lymphopoietic system, bladder, and kidney cancer mortality. Kerosene exposure increased the rate of death from several digestive-tract cancers (esophageal, stomach, pancreatic, colon, and rectal cancers) and from prostate cancer. Effect estimates for these cancers increased with duration and level of exposure and were stronger when exposure was lagged.

B.3.1.4.4.2. *Study description and comment*. This study of 3,814 white male uranium processing workers employed for at least 3 months between 1-1-1951 and 12-31-1972 at the Fernald Feed Materials Production Center in Fernald, Ohio, was of deaths as of 1-1-1990. Subjects were part of a larger cohort study of Fernald workers with potential uranium and products of uranium decay exposures that observed associations with lung cancer and lymphatic/hematopoietic cancer (Ritz, 1999b). Average length of follow-up time was 31.5 years. During this period, 1,045 deaths were observed with expected numbers of deaths based upon age- and calendar-specific U.S. white male mortality rates and age- and calendar-specific white male mortality rates from the NIOSH Computerized Occupational Referent Population System (CORPS) (Zahm, 1992). Internal analyses based upon risk-set sampling and Cox proportional hazards modeling compared workers with differing exposure intensity rankings (light and moderate) and a category for no- TCE exposure/<2 year duration TCE exposure.

Fernald produced uranium metal products for defense programs (Hornung et al., 2008). Subjects had potential exposures to uranium, mainly as insoluble compounds and varying from depleted to slight enriched, small amounts of thorium, an alpha particle emitter, respiratory irritants such as tributyl phosphate, ammonium hydroxide, sulfuric acid and hydrogen fluoride, trichloroethylene, and cutting fluids (Ritz, 1999a, b). Exposure assessment for analysis of chemical exposures utilized a job-exposure matrix (JEM) to assign intensity of TCE, cutting fluids, and kerosene to individual jobs from the period 1952 to 1977. Industrial hygienists, a plant foreman, and an engineer during the late 1970s and early 1980s determined the likelihood of exposure to TCE, cutting fluids, and kerosene for each job title and plant area. Based on work records, the workforce appeared stable and 54% were employed ≥5 years and had held only one job title during employment. Both intensity or exposure level and duration of exposure in years were used to rank subjects into 4 categories of no exposure (level 0), light exposure (level 1), moderate exposure (level 2), and heavy exposure (level 3). Seventy eight (78) percent of the cohort was identified with some potential for TCE exposure, 2,792 subjects were identified with low TCE exposure (94%), 179 with moderate exposure (6%), and no subjects were identified with heavy TCE exposure. TCE exposure was highly correlated with other chemical exposures and with alpha radiation (Ritz, 1999a, b; Hornung et al., 2008). Fernald subjects had higher exposures to radiation compared to those of radiation-exposed Rocketdyne workers (Ritz, 1999b; Ritz et al., 1999, 2000). Atmospheric monitoring information is lacking on TCE exposure conditions as is information on changes in TCE usage over time. The cohort was identified from company rosters and personnel records and it is not known whether these were sources for a subject's job title information. Analysis of TCE exposure carried out using conditional logistic regression adjusting for pay status, time since first hired, external and internal radiation dose and previous chemical exposure. Relative risks for TCE exposure are also presented with a lag time period of 15 years.

Overall, strengths of this study are the long follow-up time and a large percentage of the cohort who had died by the end of follow-up. TCE exposure intensity is low in this cohort, 94% of TCE exposed subjects were identified with "light" exposure intensity, and all subjects had potential for radiation exposure, which was highly correlated with chemical exposures. No information is presented on the definition of "light" exposure and monitoring data are lacking. Only 179 subjects were identified with TCE exposure above "light" and the number of cancer deaths not presented. The published paper reported limited information on site-specific cancer and TCE exposure; risk estimates are reported for lymphatic and hematopoietic cancers, esophageal and stomach cancer, liver cancer, prostate cancer and brain cancer. Risk estimates for bladder and kidney cancer and TCE exposure are found in NRC (2006). Few deaths were observed with moderate TCE exposure and exposure durations of longer than 2 years: 1 death due to lymphatic and hematopoietic cancer, 0 deaths due to kidney or bladder cancer (as noted in NRC, 2006), and 2 liver cancer deaths among these subjects. Low statistical power reflecting few cases with moderate TCE exposure and multicolinearity of chemical and radiation exposures greatly limits the support this study provides in an overall weight-of-evidence analysis.

Ritz B. 1999a. Cancer mortality among workers exposed to chemicals during uranium processing. J Occup Environ Med 41:556−566.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The hypothesis in this study was to examine the influence of chemical exposures in the work environment of the Fernald Feed Materials Production Center (FFMPC) in Fernald, Ohio, on cancer mortality with a focus on the effects of TCE, cutting fluids, and a combination of kerosene exposure with carbon (graphite) and other solvents.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	3,814 white male subjects identified from company rosters and personnel records, hired between 1951 and 1972 and who were employed continuously for 3 mos and monitored for radiation. 2,971 subjects identified as exposed to TCE at "light" and "moderate" exposures. Subjects were identified in a previous study of cancer mortality and radiation exposure and most subjects had radiation exposures above 10+ mSV (Ritz, 1999b). External analysis: U.S. white male mortality rates and NIOSH-Computerized Occupational Referent Population System mortality rates. Internal analysis: cohort subjects according to level and duration of chemical exposure.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality. Vital status searched through Social Security Administration records, before 1979, and National Death Index for the period 1979−1989.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	External analysis: ICDA, 8th revision. Internal analysis: aggregation of several subsite causes of deaths into larger categories based on ICD, 9th revision.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Semiquantitative approach and development of job-exposure matrix. JEM developed by expert assessment by plant employees to classify jobs into four levels of chemical exposures for the period 1952 to 1977. Intensity using the four-level scale and duration of exposure to TCE, cutting fluids and kerosene were assigned to individual cohort subjects using JEM. 73% of cohort identified as TCE exposed (2,971 male with TCE exposure in cohort of 3,814 subjects). Only 4% of TCE-exposed subjects with exposure identified as “moderate” and no subjects with “high” exposure. High correlation between TCE and other chemical exposure and radiation exposure.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	All workers without death certificate assumed alive at end of follow-up.
>50% cohort with full latency	Average follow-up time, 31.5 yrs.
Other	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,045 deaths (27% of cohort), 328 due to cancer. No information on number of all-cancer deaths among TCE exposed subjects, although reported numbers for specific sites reported by Ritz (1999a) or NRC (2006): >2 year exposure duration, hemato- and lymphopoietic cancer (*n* = 18 with light exposure, 1 with moderate exposure), esophageal and stomach cancer (*n* = 15 with light exposure, 0 with moderate exposure), liver cancers (*n* = 3 with light exposure, 1 with moderate exposure), kidney and bladder cancers, (n = 7 with light exposure, 0 with moderate exposure) prostate cancers (*n* = 10 with light exposure, 1 with moderate exposure), and brain cancers (*n* = 6 with light exposure, 1 with moderate exposure).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	External analysis: age- and calendar-specific mortality rates for white males. Internal analysis: pay status, time since first hired, and cumulative time-dependent external- and internal-radiation doses (continuous); indirect assessment of smoking through examination of smoking distribution by chemical exposure.
Statistical methods	SMR (external analysis) and RR (internal analysis).
Exposure-response analysis presented in published paper	Yes, RR presented for exposure to TCE (level 1 and level 2, separately) by duration of exposure.
Documentation of results	Adequate.

RR = relative risk.

B.3.1.4.5. *Henschler et al. (1995).*

B.3.1.4.5.1. ***Author's abstract.***

> A retrospective cohort study was carried out in a cardboard factory in Germany to investigate the association between exposure to trichloroethene (TRI) and renal cell cancer. The study group consisted of 169 men who had been exposed to TRI for at least 1 year between 1956 and 1975. The average observation period was 34 years. By the closing day of the study (December 31, 1992) 50 members of the cohort had died, 16 from malignant neoplasms. In 2 out of these 16 cases, kidney cancer was the cause of death, which leads to a standard mortality ratio of 3.28 compared with the local population. Five workers had been diagnosed with kidney cancer: four with renal cell cancers and one with an urothelial cancer of the renal pelvis. The standardized incidence ratio compared with the data of the Danish cancer registry was 7.97 (95% CI: 2.59-18.59). After the end of the observation period, two additional kidney tumors (one renal cell and one urothelial cancer) were diagnosed in the study group. The control group consisted of 190 unexposed workers in the same plant. By the closing day of the study 52 members of this cohort had died, 16 from malignant neoplasms, but none from kidney cancer. No case of kidney cancer was diagnosed in the control group. The direct comparison of the incidence on renal cell cancer shows a statistically significant increased risk in the cohort of exposed workers. Hence, in all types of analysis the incidence of kidney cancer is statistically elevated among workers exposed to TRI. Our data suggest that exposure to high concentrations of TRI over prolonged periods of time may cause renal tumors in humans. A causal relationship is supported by the identity of tumors produced in rats and a valid mechanistic explanation on the molecular level.

B.3.1.4.5.2. *Study description and comment*. This was a cohort study of workers in a cardboard factory in the area of Arnsberg, Germany. Trichloroethylene was used in this area until 1975 for degreasing and solvent needs. Plant records indicated that 2,800−23,000 L/year was used. Small amounts of tetrachloroethylene and 1,1,1-trichloroethane were used occasionally, but in much smaller quantities than trichloroethylene. Trichloroethylene was used in three main areas: cardboard machine, locksmith's area, and electrical workshop. Cleaning the felts and sieves and cleaning machine parts of grease were done regularly every 2 weeks, in a job that required 4−5 hours, plus whatever additional cleaning was needed. Trichloroethylene was available in open barrels and rags soaked in it were used for cleaning. The machines ran hot (80−120°C) and the cardboard machine rooms were poorly ventilated and warm (about 50°C), which would strongly enhance evaporation. This would lead to very high concentrations of airborne trichloroethylene. Cherrie et al. (2001) estimated that the machine cleaning exposures to trichloroethylene were greater than 2,000 ppm. Workers reported frequent strong odors and a sweet taste in their mouths. The odor threshold for trichloroethylene is listed as 100 ppm

(ATSDR, 1997). Workers often left the work area for short breaks "to get fresh air and to recover from drowsiness and headaches." Based on reports of anesthetic effects, it is likely that concentrations of trichloroethylene exceeded 200 ppm (Stopps and McLaughlin, 1967). Those reports, the work setting description, and the large volume of trichloroethylene used are all consistent with very high concentrations of airborne trichloroethylene. The workers in the locksmith's area and the electrical workshop also had continuous exposures to trichloroethylene associated with degreasing activities; parts were cleaned in cold dip baths and left on tables to dry. Trichloroethylene was regularly used to clean floors, work clothes, and hands of grease, in addition to the intense exposures during specific cleaning exercises, which would produce a background concentration of trichloroethylene in the facility. Cherrie et al. (2001) estimated the long-term exposure to trichloroethylene was approximately 100 ppm.

The subjects in this study clearly had substantial peak exposures to trichloroethylene that exceeded 2,000 ppm and probably sustained long-term exposures greater than 100 ppm, which are not confounded by concurrent exposures to other chlorinated organic solvents.

Henschler D, Vamvakas S, Lammert M, Dekant W, Kraus B, Thomas B, Ulm K. 1995. Increased incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethene. Arch Toxicol 69:291−299.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract "…retrospective cohort study was carried out in a cardboard factory I Germany to investigate the association between exposure to trichloroethene and renal cell cancer."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Employee records were used to identify 183 males employed in a cardboard factory for at least 1 yr between 1956 and 1975 and with presumed TCE exposure and a control group of 190 male workers at same factory during the same period of time but in jobs not involving possible TCE exposure. Mortality rates from German population residing near factory used as referent in mortality analysis. Renal cancer incidence rates from Danish Cancer Registry used to calculate expected number of incident cancer. The age-standardized rate in the late 1990s among men in Denmark was 10.6 and in Germany it was 1.2 (Ferlay, 2004). If these differences in rates apply when the study was carried out, this would imply that the expect number of deaths would have been inflated by about 14% (and the rate ratio underestimated by that amount).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality and renal cell cancer incidence.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-9 for deaths. Hospital pathology records were used to verify diagnosis of renal cell carcinoma.

Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Walkthrough survey and interviews with long-term employees were used to identify work areas and jobs with potential TCE exposure. The workers in the locksmith's area and the electrical workshop also had continuous exposures to trichloroethylene associated with degreasing activities; parts were cleaned in cold dip baths and left on tables to dry. **Cherrie et al. (2001) estimated that the machine cleaning exposures to trichloroethylene were greater than 2,000 ppm with average long-term exposure as 10−225 ppm. Estimated average chronic exposure to TCE was ~100 ppm to subjects using TCE in cold degreasing processes.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	14 exposed subjects (8%) were excluded from life-table analysis and no information is presented in paper on loss-to-follow-up among control subjects.
>50% cohort with full latency	Median follow-up period was over 30 yrs for both exposed and control subjects.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	50 total deaths (30%) and 15 cancer death among exposed subjects. 52 deaths (27%) and 15 cancer deaths among control subjects.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and calendar-year.
Statistical methods	SMR and SIR. Analysis excludes person-years of subjects excluded from exposed population with the number of person-years underestimated and an underestimate of the expected numbers of deaths and incident renal carcinoma cases.

Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

SIR = standardized incidence ratio.

B.3.1.4.6. *Greenland et al. (1994).*

B.3.1.4.6.1. ***Author's abstract.***

> To address earlier reports of excess cancer mortality associated with employment at a large transformer manufacturing plant each plant operation was rated for seven exposures: Pyranol (a mixture of polychlorinated biphenyls and trichlorobenzene), trichloroethylene, benzene, mixed solvents, asbestos, synthetic resins, and machining fluids. Site-specific cancer deaths among active or retired employees were cases; controls were selected from deaths (primarily cardiovascular deaths) presumed to be unassociated with any of the study exposures. Using job records, we then computed person-years of exposure for each subject. All subjects were white males. The only unequivocal association was that of resin systems with lung cancer (odds ratio = 2.2 at 16.6 years of exposure, P = 0.0001, in a multiple logistic regression including asbestos, age, year of death, and year of hire). Certain other odds ratios appeared larger, but no other association was so robust and remained as distinct after considering the multiplicity of comparisons. Study power was very limited for most associations, and several biases may have affected our results. Nevertheless, further investigation of synthetic resin systems of the type used in the study plant appears warranted.

B.3.1.4.6.2. ***Study discussion and comment*.** This nested case-control study at General Electric's Pittsfield, MA, plant was of deaths reported to the GE pension fund among employees vested in the pension fund. The cohort from which cases and controls were identified was defined as plant employees who worked at the facility before 1984; whose date of deaths was between 1969, the date pension records became available, and 1984; and existence of a job history record. The size of the underlying employee cohort was unknown because work history records did not exist for a large fraction of former employees, especially in the earlier years of deaths. All deaths were identified from records maintained by GE's pension office; other record sources such as the Social Security Administration and National Death Index were not utilized. Requirements for eligibility or "vestment" for a pension varied over time, but for most of the study period, required 10 to 15 years employment with the company. The analysis was restricted to white males because of few deaths among females and nonwhite males. A total of 1,911 deaths were identified from pension records and cases and controls, with 90 deaths excluded as possible cases and controls due to several reasons. Cases were identified as site-specific deaths and controls were selected from the remaining noncancer deaths due to circulatory disease, respiratory disease, injury, and other causes. No information was available on the number of controls selected per case. Controls were not matched to cases, were slightly older than cases, and were from earlier birth cohorts which have a lower job history availability or greater frequency of missing exposure ratings in work history records (Salvan, 1990). Statistical analysis of the data included covariates for age and year of death.

The company's job history record served as the source for exposure rating. The JEM linked possible exposures to over 1,000 job title from 50 separate departments and 100 buildings. A categorical ranking was developed for exposure to seven exposures (Pyranol, TCE, benzene, other solvents, asbestos, resin systems, machining fluids) from 1901 to 1984 based upon on-site interviews with 18 long-term employees and knowledge of one of the study investigators who was an industrial hygienist. Two categories were used for potential TCE exposure: Level 1, duration of indirect exposure (TCE in workplace but does not work directly with TCE) and Level 2, duration of direct work with TCE, with the continuous exposure scores rescaled to the 97th percentile of controls (Salvan, 1990). Statistical analyses in Greenaland et al. (1994) collapsed these two categories into a dichotomous ranking of no exposure or any exposure. In many instances, exposure levels were inaccurately estimated and some exposures were highly correlated (Salvan, 1990). Although of low correlation, TCE exposure was statistically significantly correlated with exposure to other solvents ($r = 0.11$), benzene ($r = 0.22$) and machining fluids ($r = 0.28$) (Salvan, 1990). Industrial hygiene monitoring data were not available before 1978 and limited production and purchase records did not extend far back in time (Salvan, 1990). TCE was used as a degreaser since the 1930s and discontinued between 1966 and 1975, depending on department. In all, fewer than 10% of jobs were identified as have TCE exposure potential, primarily through indirect exposure and not directly working with TCE. In fact, few subjects were identified with as working directly with TCE (Salvan, 1990). It is not surprising that exposure score distributions were highly skewed towards zero (Salvan, 1990). No details were provided on the protocol for processing the jobs in the work histories into job classifications.

Job history information was missing for roughly 35% of the cases and controls, particularly from subjects with earlier years of death. The highest percentage of missing information among cases was for leukemia deaths (43% of deaths) and the lowest percentage for rectal deaths (11%). Moreover, work history records did not exist for a large fraction of former employees, especially in the earlier years of death. Bias resulting from exposure misclassification is likely high due to the lack of industrial monitoring to support rankings and the inability of the JEM to account for changes in TCE exposure concentrations over time.

This study had a number of weaknesses with the likely result of dampening observed risks. Deaths were underestimated given nonpensioned employees are not included in the analysis; possible differences in exposure potential between pensioned and nonpensioned workers may introduce bias, particularly if a subject leaves work as a consequence of a precondition related to exposure, and would dampen observed associations (Robins and Blevins, 1987). Misclassification bias related to exposure is highly likely given missing job history records for over one-third of deaths, mostly among deaths from the earlier study period, a period when TCE was used. Salvan (1990) noted "exposure measurements should be regarded as heavily nondifferentially misclassified relative to the true exposure does" and exposure associations with outcomes will be underestimated. For TCE specifically, the development of exposure assignments in this study was insensitivity to define TCE exposures of the cohort-industrial hygiene data were not available for the time period of TCE use, exposure rates applied to a job-building-operation time matrix and may not reflect individual variation, and exposure ratings obtained by employee interview are subject to subjective assessment and measurement error. NRC (2006) also noted a low likelihood of exposure potential to subjects in this nested case-control study. Overall, the sensitivity of this study for evaluating cancer and TCE exposure is quite limited. The inability of this study to detect associations for two known human carcinogens, benzene and leukemia and asbestos and lung cancer, provides ancillary support for the study's low sensitivity and statistical power.

Greenland S, Salvan A, Wegman DH, Hallock MF, Smith TH. 1994. A case-control study of cancer mortality at the transformer-assembly facility. Int Arch Occup Environ Health 66:49−54.

Greenland S. 1992. A semi-Bayes approach to the analysis of correlated multiple associations with an application to an occupational cancer-mortality study. Stat Med 11:219−230.

Salvan A. 1990. Occupational exposure and cancer mortality at an electrical manufacturing plant: A case-control study. Ph.D. Dissertation, University of California, Los Angeles.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The study was carried out to reevaluate an earlier observation from a PMR study of GE employment and excess leukemia and colorectal cancer risks.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Selection of cases and controls is not adequate because only deaths among pensioned workers were included in the analysis. Also, the size of the underlying cohort was not known and potential for selection bias is likely given cases and controls are drawn from a select population. Cases were identified from deaths among white males employed before 1984, who had died between 1969 and 1984, and for whom a job history record was available. Controls selected from noncancer deaths due to cardiovascular disease, circulatory disease, respiratory disease, injury, or other causes. Controls are not matched to cases on covariates such as age, or date of hire. In total, 2,653 subjects were identified as meeting criteria for inclusion in subject, either as a case or as a control. Job history records were available for 1,714 (512 cases, 1,202 controls) of these subjects (65%).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICDA, 8th revision.
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Dichotomous ranking, not exposed/exposed, for indirect and direct exposure potential. Most subjects identified with indirect TCE exposure. The company's job history record served as the source for exposure rating. The JEM linked possible exposures to over 1,000 job title from 50 separate departments and 100 buildings. Potential TCE exposure assigned to 10% of all job titles. The seven exposures were highly correlated. NRC (2006) noted a low likelihood of TCE exposure potential to subjects in this nested case-control study.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	Record study.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	220 of 732 cases and 1,202 or 1,921 possible controls had job history records; job history records are missing for 35% of all possible cases and controls. Any potential TCE exposure prevalence among cases: Laryngeal, pharyngeal cancer, 38% Liver and biliary passages, 22% Pancreas, 45% Lung, 33% Bladder, 30% Kidney, 33% Lymphoma, 27% Leukemias, 36% Brain, 31% Control exposure prevalence, 34%.
Control for potential confounders in statistical analysis	Age and year of death. Other unidentified covariates are included if risk estimate is altered by more than 20%.
Statistical methods	Logistic regression with (1) dichotomous exposure (Greenland, 1994) (2) continuous exposure (Salvan, 1990), (3) epoch analysis (Salvan, 1990), and (4) empirical bayes models (Greenland, 1992).
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

B.3.1.4.7. *Sinks et al. (1992).*

B.3.1.4.7.1. ***Author's abstract.***

> A physician's alert prompted us to investigate workers' can cancer risk at a paperboard printing manufacturer. We conducted a retrospective cohort mortality study of all 2,050 persons who had worked at the facility for more than 1 day, calculated standardized incidence ratios (SIRs) for bladder and renal cell cancer, and conducted a nested case-control study for renal cell cancer. Standardized mortality ratios (SMRs) from all causes [SMR = 1.0, 95% confidence interval (CI) = 0.9 – 1.2] and all cancers (SMR = 0.6, 95% CI = 0.3 – 1.0) were not greater than expected. One bladder cancer and one renal cell cancer were included in the mortality analysis. Six incident renal cell cancers were observed, however, compared with less than two renal cell cancers expected (SIR = 3.7, 95% CI = 1.4 – 8.1). Based on a nested case-control analysis, the risk of renal cell carcinoma was associated with overall length of employment but was not limited to any single department or work process. Although pigments containing congeners of dichlorobenzidine and o-toluidine had been used at the plant, environmental sampling could not confirm any current exposure. Several limitations and a potential selection bias limit the inferences that can be drawn.

B.3.1.4.7.2. ***Study description and comment***. Sinks et al. (1992) is the published report of analyses examining morbidity and mortality among employees at a James River Corporation plant in Newnan, GA. This plant manufactured paperboard (cardboard) packaging. The study was carried out as a National Institute of Occupational Safety and Health, Health Hazard Evaluation to investigate a possible cluster of urinary tract cancers and work in the plant's Finishing Department (NIOSH, 1992). A cohort of 2,050 white and nonwhite, male and female, subjects were identified from company personnel and death records, considered complete since 1-1-1957, and were follows for site-specific mortality and cancer morbidity to 6-30-1988. Records of an additionally 36 subjects were missing hire dates or birth dates, indicated employment duration of less than 1 day, and or employment outside the study period and these subjects were excluded from the analysis. This study suffers from missing information. A large percentage of personnel records did not identify a subject's race and these subjects were considered as white in statistical analyses. Additionally, vital status was unknown for approximately 10% of the cohort. Life-table analyses are based upon U.S. population age-, race-, sex-, calendar- and cause-specific mortality rates. Expected numbers of incident bladder and kidney cancers for white males were derived using white male age-specific bladder and renal cell incidence rates from the Atlanta-Surveillance, Epidemiology, and End Results (SEER) registry for the years 1973 to 1977.

A nested case-control analysis of the incident renal carcinoma cases was also undertaken. This analysis is based on 6 renal cell carcinoma cases and 48 controls (1:8 matching) who were selected by risk set sampling of all employees born within 5 years of the case, the same sex as the case, and having attained the age at which the case was diagnosed or died if date of diagnosis was not known. A diagnosis of renal carcinoma was confirmed for 4 of the 6 cases through pathologic examination. Both the nested case-control analysis and the life-table analyses of morbidity included a renal carcinoma case from the original cluster.

Exposures are poorly defined in this study assessing renal cancer among paper board printing workers. Trichloroethylene was mentioned in material-safety data sheets for one or more materials used by the process but no information was provided regarding TCE usage and use by job title. It was not possible to assess the degree of contact with trichloroethylene or the printing inks which were identified as containing benzidine. Furthermore, the lack of monitoring data precludes evaluation of possible exposure intensity. This study is limited for assessing risks associated with exposures to trichloroethylene due to the large percentage of missing information and due to its exposure assessment approach.

Sinks T, Lushniak B, Haussler BJ, Sniezek J, Deng J-F, Roper P, Dill P, Coates R. 1992. Renal cell cancer among paperboard printing workers. Epidemiol 3:483−489.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The purpose of the cohort and nested case-control investigations was to determine whether an excess of bladder or renal cell cancer had occurred among workers in a paperboard packaging plant and, if so, to determine whether it was associated with any specific exposure or work-related process.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort analysis: 2 050 males and females employed at the plant between 1-1-1957 and 6-30-1988. External referents for mortality analysis were age-, sex-, race-, and calendar- cause specific mortality rates of the U.S. population. External referents for morbidity analysis were age-specific bladder and renal-cell cancer rate for white males from the Atlanta-SEER registry for the years 1973−1977. Nested case-control analysis: Cases were all subjects with renal cell cancer; 8 nonrenal cell carcinoma controls chosen from a risk set of all employees matched to case on date of birth (within 5 yrs), sex and attained age of cancer diagnosis or death, if diagnosis date unknown.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD revision in effect at the time of death; incident cases of renal cell carcinoma diagnoses confirmed with pathology reports for 4 of 6 cases.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure in cohort analysis defined broadly at level of the plant and, in case-control study, department worked as identified on company's personnel.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	Yes, 10% of cohort with unknown vital status (*n* = 204). P-Y for these workers were censored at the date of last follow-up.

>50% cohort with full latency	18 yr average follow-up.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Department assignment based on company personnel records.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	141 total deaths (7% of cohort had died by end of follow-up), 16 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Mortality analysis: Age, race, sex, and calendar year. Morbidity analysis limited to white males: age. Nested case-control analysis: Risk set sampling matching controls to cases on date of birth (within 5 yrs), sex, and attained age at diagnosis.
Statistical methods	SIR. Conditional logistic regression used for nested case-control analysis.
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

SIR = standardized incidence ratio.

B.3.1.4.8. *Blair et al. (1989).*

B.3.1.4.8.1. ***Author's abstract.***

Work history records and fitness reports were obtained for 1 767 marine inspectors of the U.S. Coast Guard between 1942 and 1970 and for a comparison group of 1 914 officers who had never been marine inspectors. Potential exposure to chemicals was assessed by one of the authors (RP), who is knowledgeable about marine inspection duties. Marine inspectors and noninspectors had a deficit in overall mortality compared to that expected from the general U.S. population (standardized mortality ratios [SMRs = 79 and 63, respectively]). Deficits occurred for most major causes of death, including infectious and parasitic diseases, digestive and urinary systems, and accidents. Marine inspectors had excesses of cirrhosis of the liver (SMR = 136) and motor vehicle accidents (SMR = 107, and cancers of the lymphatic and hematopoietic system (SMR = 157, whereas noninspectors had deficits for these causes of death. Comparison of mortality rates directly adjusted to the age distribution of the inspectors and noninspectors combined also demonstrated that mortality for these causes of death was greater among inspectors than noninspectors (directly adjusted ratio ratios of 190, 145, and 198) for cirrhosis of the liver, motor vehicle accidents, and lymphatic and hematopoietic system cancer, respectively. The SMRs rose with increasing probability of exposure to chemicals for motor vehicle accidents, cirrhosis of the liver, liver cancer, and leukemia, which suggests that contact with chemicals during inspection of merchant vessels may be involved in the development of these diseases among marine inspectors. physician's alert prompted us to investigate workers' can cancer risk at a paperboard printing manufacturer. We conducted a retrospective cohort mortality study of all 2,050 persons who had worked at the facility for more than 1 day, calculated standardized incidence ratios (SIRs) for bladder and renal cell cancer, and conducted a nested case-control study for renal cell cancer. Standardized mortality ratios (SMRs) from all causes [SMR = 1.0, 95% confidence interval (CI) = 0.9 – 1.2] and all cancers (SMR = 0.6, 95% CI = 0.3 – 1.0) were not greater than expected. One bladder cancer and one renal cell cancer were included in the mortality analysis. Six incident renal cell cancers were observed, however, compared with less than two renal cell cancers expected (SIR = 3.7, 95% CI = 1.4 – 8.1). Based on a nested case-control analysis, the risk of renal cell carcinoma was associated with overall length of employment but was not limited to any single department or work process. Although pigments containing congeners of dichlorobenzidine and o-toluidine had been used at the plant, environmental sampling could not confirm any current exposure. Several limitations and a potential selection bias limit the inferences that can be drawn.

B.3.1.4.8.2. ***Study description and comment***. This cohort of 1,767 U. S. Coast Guard male officers and enlisted personnel performing marine inspection duties between 1942 and 1970 and 1,914 noninspectors matched to inspectors for registry, rank and year that rank was achieved examined mortality as of January 1, 1980. Standardized mortality ratios compared the observed number of site-specific deaths among marine inspectors (n = 483, 27%) to that expected of the total U. S. white male population and to standardized mortality ratios of noninspectors (n = 369, 19%). The cohort was predominantly white (91%), race was unknown for the remaining 8% of subjects, considered in the statistical analysis as white, with a large percentage (69%) of the marine inspectors having >20 year employment duration. The minimum latent period was 10 years, calculated from the end date of cohort identification to the date of vital status ascertainment.

This study lacks exposure information on potential exposures of marine inspectors, who enter cargo tanks, void spaces, cofferdams, and pump rooms during inspections. TCE is identified in the paper as a possible exposure along with nine other agents. One authors acquainted with Coast Guard processes estimated the level of exposure to general chemical exposures during a marine inspection. A four-point rating scales was developed: nonexposed, person generally held administrative position; low exposed, assigned to staff with duties that occasionally required vessel inspections; moderate exposed, assign to inspection duties that did not regularly include hull structures, and regular inspection of hull structures in geographic areas where chemicals were not major items of cargo; and, high exposed, assigned to subjects who performed hull inspections at ports were vessels transported chemicals. A cumulative exposure score was calculated by summing the product of the four-point rating scale and the duration in each job.

Overall, the exposure assessment in this study is insufficient for examining TCE exposure and cancer mortality. Furthermore, the few site-specific deaths among marine inspectors greatly limits statistical power.

Blair A, Haas T, Prosser R, Morrissette M, Blackman, Grauman D, van Dusen P, Morgan F. 1989. Mortality among United States Coast Guard marine Inspectors. Arch Environ Health 44:150-156.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The purpose of the cohort study was to examine mortality patterns among Coast Guard marine inspectors. This study was not designed to examine specific exposures, including TCE.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,767 U. S. Coast Guard male officers and enlisted personnel performing marine inspections between 1942 and 1970 and 1,914 noninspectors matched to inspectors on registry, rank, and year that rank was achieved. External referents: age-specific mortality rates of the U. S. white male population and noninspectors.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICDA, 8th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	TCE identified in paper as one of ten potential exposures; however, no exposure assessment to TCE to individual subjects. Exposure in cohort analysis defined broadly at level of the plant and, in case-control study, department worked as identified on company's personnel. A cumulative exposure surrogate developed from duration in each job and a four-point rating scale: nonexposed, person generally held administrative position; low exposed, assigned to staff with duties that occasionally required vessel inspections; moderate exposed, assign to inspection duties that did not regularly include hull structures, and regular inspection of hull structures in geographic areas where chemicals were not major items of cargo; and, high exposed, assigned to subjects who performed hull inspections at ports were vessels transported chemicals.
CATEGORY D: FOLLOW-UP (COHORT)	

More than 10% loss to follow-up	No
>50% cohort with full latency	Not reported; minimum latent period was 10 years.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	483 deaths among marine inspectors (27% of cohort), 103 cancer deaths.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Mortality analysis: Age, race, sex, and calendar year. Directly adjusted rate ratios compared cause-specific SMR of marine inspectors to that of noninspectors.
Statistical methods	SMR and RR.
Exposure-response analysis presented in published paper	Yes, using a ranked cumulative exposure surrogate.
Documentation of results	Adequate.

RR = relative risk. SMR = standardized mortality ratio.

B.3.1.4.9. *Shannon et al. (1988).*

B.3.1.4.9.1. ***Author's abstract.***

> A historical prospective study of cancer in lamp manufacturing workers in one plant was conducted. All men and women who worked for a total of at least 6 months and were employed at some time between 1960 and 1975 were included. Work histories were abstracted and subjects were divided according to whether they had worked in the coiling and wire drawing area (CWD). Cancer morbidity from 1964 to 1982 was ascertained via the provincial registry, and was compared with the site-specific incidence in Ontario, adjusting for age, sex and calendar period. Of particular interest were primary breast and gynecological cancers in women.
>
> The cancers of a priori concern were significantly increased in women in CWD, but not elsewhere in the plant. The excess was greatest in those with more than 5 yr exposure (in CWD) and more than 15 yr since first working in CWD, with eight cases of breast and gynecological cancers observed in this category compared with 2.67 expected. Only three cancers occurred in men in CWD. Environmental measurements had not been made in the past and little information was available on substances used in the 1940s and 1950s, the period when the women with the highest excess began employment. It is known that methylene chloride and trichloroethylene have been used, but not enough is known about the dates and patterns.

B.3.1.4.9.2. ***Study description and comments***. This cohort of 1,770 workers (1,044 females, 826 males) employed >6 months and working between 1960 and 1975 at a General Electric plant in Ontario, Canada, in the lamp manufacturing department identified cancer incidence cases from a regional cancer registry from 1964, the first date of high quality information, to 1982. Office workers were included in the study population. The study was carried out in response to previous reports of excess breast and gynecological cancer in women employed in the CWD area. Standardized incidence ratios (SIR) compared the observed number of site-specific incident cancers to that expected of the Ontario population and supplied by the regional cancer registry. SIR estimates were calculated for all lamp department workers, and for two subgroups defined by job title, workers in the coil and wire-drawing area (CWD) and workers in all other areas. The cohort was successfully traced, with low rates of lost to follow-up (6% among CWD workers, 7 all other workers). A total of 98 incident cancer cases were identified (58 in females, 40 in males) and over half of the incident cancers in females (n = 31) due to breast and gynecological cancers. The number of incident cancers is likely underestimated given the 4-year period between cohort identification and the first date of high quality information in the cancer registry. Additionally, cancer cases among workers who moved from the province would not be found in the registry, leading to underascertainment of cases. This is likely a small number given follow-up tracing identified 2% of workers had left the province.

This study lacks exposure information on individual study subjects. Exposures in CWD were of concern given previous reports. The study lacks exposure monitoring data and potential exposures in CWD area were identified using purchase records. A number of chemicals were identified including methylene chloride from 1959 onward and trichloroethylene, which records suggested may have been used beforehand.

Overall, the exposure assessment in this study is insufficient for examining TCE exposure and cancer mortality. The inclusion of office workers, who likely have low potential exposure, would introduce a downward bias. Furthermore, the few site-specific deaths among CWD and all other workers greatly limits statistical power.

Shannon HS, Haines T, Bernholz C, Julian JA, Verma DK, Jamieson E, Walsh C. 1988. Cancer morbidity in lamp manufacturing workers. Am J Ind Med 14:281-290.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was undertaken in response to previous report of apparent excess breast and gynecological cancers in women employed in the coil and wire drawing area of a lamp manufacturing plant.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cohort analysis: 1,770 workers (1,044 females, 826 males)in the lamp manufacturing department of a GE plant in Ontario Province, Canada. External referents: Age-, sex- and race-specific site-specific cancer incidence rates for Ontario Province population
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not reported.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This study does not assign TCE exposure to individual subjects. Job title and work in the CWD area used to assign exposure potential and chemical usage in CWD identified from purchase records. Methylene chloride used from 1959 onward, with one report from 1955 indicating TCE used as degreasing solvent.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No, follow-up was complete for 6% of CWD workers and 7% for all other workers.
>50% cohort with full latency	Not reported
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	

>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	98 incident cancer cases
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, race, sex, and calendar year.
Statistical methods	SIR.
Exposure-response analysis presented in published paper	No.
Documentation of results	Adequate.

CWD = coil and wire drawing area. SIR = standardized incidence ratio.

B.3.1.4.10. *Shindell and Ulrich (1985).*

B.3.1.4.10.1. ***Author's abstract.***

> A prospective study was conducted of 2,646 employees who worked three months or more during the period January, 1957, through July, 1983, in a manufacturing plant that used trichloroethylene as a degreasing agent throughout the study period. Ninety-eight percent of the study cohort were traced; they accounted for 16,388 person-years of employment and 38,052 person-years of follow-up. Mortality experience was found to be generally more favorable than that of the comparable segment of the U.S. population over the same period of time. For the white male cohort there were fewer deaths than expected from heart disease, cancer, and trauma (standard mortality rate for all causes = 0.79, p less than .01). Reports by current and former employees of health problems requiring medical treatment showed that there were only one third as many persons with heart disease or hypertension as were reported in a comparable reference population studied over the past five years.

B.3.1.4.10.2. ***Study description and comment***. This study of 2, 546 current and former office and production employees at a manufacturing plant in northern Illinois compares broad groupings of cause-specific mortality between 1957 and 1983 to expected number of deaths based on U.S. population mortality rates for the period. The published paper lacks an assessment of TCE exposure other than noting TCE was used as a degreasing agent at the plant. No information is presented on quantity used, job titles with potential exposure, or likely exposure concentrations Not all study subjects had the same potential for exposure and the inclusion of office workers who had a very low exposure potential decreased the study's detection sensitivity. Deaths were identified from company records or from direct or indirect contact with former employees or next-of-kin for subjects not known to the company to be deceased instead of using national-based registries such as Social Security listings or National Death Index for identifying vital status. There were few deaths in this cohort, a total of 141 among male and female subjects; vital status could not be ascertained for 52 subjects. The few numbers of cancer deaths (21 total) precluded examination of cause-specific cancer mortality. Overall, this study provides no information on TCE and cancer; it lacked exposure assessment to TCE and the few cancer deaths observed greatly limited its detection sensitivity.

Shindell S, Ulrich S. 1985. A cohort study of employees of a manufacturing plant using trichloroethylene. J Occup Med 27:577−579.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was designed to assess mortality patterns of office and production employees at an Illinois manufacturing plant.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	2,646 males and female workers employed from 1-1-1957 to 7-31-1983. Mortality rates of U.S. population used as referent. The paper lacks information on source for identifying cohort subjects and if company records were complete.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	The paper does not identify TCE usage other than as a degreaser. Conditions of exposure and jobs potentially exposure are not identified in paper. This study lacks an assessment of TCE exposure.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	2%.
>50% cohort with full latency	No information provided in paper.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	This study does not use standard approaches to verify deaths and vital status. Deaths are self-reported in response to contact by employer representative. 141 deaths (6%) were reported to employer, 9 deaths lacked a death certificate.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Sex and race.
Statistical methods	SMR.
Exposure-response analysis presented in published paper	No.
Documentation of results	The paper lacks discussion of process used to contact former employees to verify vital status and methods used to identify subjects.

B.3.1.4.11. *Wilcosky et al. (1984).*

B.3.1.4.11.1. ***Author's abstract.***

> Some evidence suggests that solvent exposures to rubber industry workers may be associated with excess cancer mortality, but most studies of rubber workers lack information about specific chemical exposure. In one large rubber and tire-manufacturing plant, however, historical documents allowed a classification of jobs based on potential exposures to all solvents that were authorized for use in the plant. A case-control analysis of a 6,678 member cohort compared the solvent exposure histories of a 20% age-stratified random sample of the cohort with those of cohort members who died during 1964-1973 for stomach cancer, respiratory system cancer, prostate cancer, lymphosarcoma, or lymphatic leukemia. Of these cancers, only lymphosarcoma and lymphatic leukemia showed significant positive associations with any other potential solvents exposures. Lymphatic leukemia was especially strongly related to carbon tetrachloride (OR = 1.3, p< .0001) and carbon disulfide (OR = 8.9, p = .0003). Lymphosarcoma showed similar, but weaker, association with these two solvents. Benzene, a suspected carcinogen, was not significantly associated with any of the cancers.

B.3.1.4.11.2. *Study description and comment*. Exposure was assessed in this nested case-control study of four site-specific cancers among rubber workers at a plant in Akron, OH through use of a JEM originally used to examine benzene specifically, but had the ability to assess 24 other solvents, including TCE, or solvent classes. Exposure was inferred using information on production operations and product specifications that indicated whether solvents were authorized for use during tire production, and by process area and calendar year. A subject's work history record was linked to the JEM to assign exposure potential to TCE. Overall, a low prevalence of TCE exposure, ranging from 9 to 20% for specific cancers was observed among cases.

The JEM was developed originally to assign exposure to benzene and other aromatic solvents in a nested case-control study of lymphocytic leukemia (Arp et al., 1983). Details of exposure potential to TCE are not described by either Arp et al. (1983) or Wilcosky et al. (1984). No data were provided on the frequency of exposure-related tasks. Without more information, it is not possible to determine the quality of some of the assignments. Similarly, the lack of industrial hygiene monitoring data precluded validation of the JEM.

Cases of respiratory, stomach and prostate cancers; lymphosarcoma and reticulum cell sarcoma; and lymphatic leukemia were identified from a previous study which had observed associations with these site-specific cancers among a cohort of rubber workers employed at a large tire manufacturing plant in Akron, OH. Statistical power is low in this study, particularly for evaluation of lymphatic cancer for which there were 9 cases of lymphosarcoma and 10 cases of lymphatic leukemia. Controls were chosen from a 20% age-stratified random sample of the cohort. The published paper does not identify if subjects with other diseases associated with solvents or TCE were excluded as controls. If no exclusion criteria were adopted, a bias may have been introduced which would dampen observed associations towards the null.

The few details provided in the paper on exposure assessment and JEM developments, few details of control selection, the low prevalence of TCE exposure and the few lymphatic cancer cases greatly limit the ability of this study for assessing risks associated with exposures to trichloroethylene.

Wilcosky TC, Checkoway H, Marshall EG, Tyroler HA. 1984. Cancer mortality and solvent exposure in the rubber industry. Am Ind Hyg Assoc J 45:809−811.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was identified as “exploratory” to examine several site-specific cancer and specific solvents, primarily benzene.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Underlying population at risk was a cohort of 6,678 male workers employed in the rubber industry in 1964. Cases are deaths due to respiratory, stomach and prostate cancers; lymphosarcoma; and lymphatic leukemia observed in the cohort analysis—30 deaths due to stomach cancer, 333 deaths from prostate cancer, 9 deaths from lymphosarcoma, and 10 deaths from lymphatic leukemia. Controls were a 20% age-stratified random sample of the cohort (exclusion criteria not identified in paper).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin’s lymphoma	ICDA, 8th revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Procedure to assign TCE and other solvent exposures based upon JEM developed originally to assess benzene and other solvent exposures (Arp et al., 1983). The JEM was linked to a detailed work history as identified from a subject’s personnel record to assign TCE exposure potential. Details of JEM for TCE not well-described in Wilcosky et al. (1984). Multiple solvent exposures likely (McMichael et al., 1976).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study with exposure assignment using JEM and personnel records.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	N/A
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE exposure prevalence: Stomach cancer, 5 exposed cases (17% exposure prevalence) Prostate cancer, 3 exposed cases (9% exposure prevalence) Lymphosarcoma, 3 exposed cases (33% exposure prevalence) Lymphatic leukemia, 2 exposed cases (20% exposure prevalence). No information presented in paper on exposure prevalence among control subjects.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age.
Statistical methods	Not described in published paper.
Exposure-response analysis presented in published paper	No.
Documentation of results	Methods and analyses not fully described in published paper.

B.3.2. Case-Control Studies

B.3.2.1. *Bladder Cancer Case-Control Studies*

B.3.2.1.1. *Pesch et al. (2000a).*

B.3.2.1.1.1. ***Author's abstract.***

> BACKGROUND: This multicentre population-based case-control study was conducted to estimate the urothelial cancer risk for occupational exposure to aromatic amines, polycyclic aromatic hydrocarbons (PAH), and chlorinated hydrocarbons besides other suspected risk factors. METHODS: In a population-based multicentre study, 1035 incident urothelial cancer cases and 4298 controls matched for region, sex, and age were interviewed between 1991 and 1995 for their occupational history and lifestyle habits. Exposure to the agents under study was self-assessed as well as expert-rated with two job-exposure matrices and a job task-exposure matrix. Conditional logistic regression was used to calculate smoking adjusted odds ratios (OR) and to control for study centre and age. RESULTS: Urothelial cancer risk following exposure to aromatic amines was only slightly elevated. Among males, substantial exposures to PAH as well as to chlorinated solvents and their corresponding occupational settings were associated with significantly elevated risks after adjustment for smoking (PAH exposure, assessed with a job-exposure matrix: OR = 1.6, 95% CI: 1.1-2.3, exposure to chlorinated solvents, assessed with a job task-exposure matrix: OR = 1.8, 95% CI: 1.2-2.6). Metal degreasing showed an elevated urothelial cancer risk among males (OR = 2.3, 95% CI: 1.4-3.8). In females also, exposure to chlorinated solvents indicated a urothelial cancer risk. Because of small numbers the risk evaluation for females should be treated with caution. CONCLUSIONS: Occupational exposure to aromatic amines could not be shown to be as strong a risk factor for urothelial carcinomas as in the past. A possible explanation for this finding is the reduction in exposure over the last 50 years. Our results strengthen the evidence that PAH may have a carcinogenic potential for the urothelium. Furthermore, our results indicate a urothelial cancer risk for the use of chlorinated solvents.

B.3.2.1.1.2. ***Study description and comment***. This multicenter study of urothelial (bladder, ureter, and renal pelvis) and renal cell carcinoma in Germany included the five regions (West Berlin, Bremen, Leverkusen, Halle, Jena), identified two case series from participating hospitals, 1,035 urothelial cancer cases and 935 renal cell carcinoma cases with a single population control series matched to cases by region, sex, and age (1:2 matching ratio to urothelial cancer cases and 1:4 matching ratio to renal cell carcinoma cases). Findings in Pesch et al. (2000a) are from analyses of urothelial cancer analysis and Pesch et al. (2000b) from analyses of renal cell carcinoma. In all, 1,035 (704 males, 331 females) urothelial carcinoma cases were interviewed face-to-face using with a structured questionnaire in the hospital within 6 months of first diagnosis and 4,298 randomly selected population controls were interviewed at home. Logistic regression models were fit separately to for males and females conditional on age (nine 5-year groupings), study region, and smoking, to examine occupational chemical exposures and urothelial carcinoma.

Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. This approach was the same as that described for the renal cell carcinoma analysis of Pesch et al. (2000b). Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, trichloroethylene, and tetrachloroethylene) to evaluate TCE potential using a JTEM. A category of "any use of a solvent" mixes the large number with infrequent slight contact with the few noted earlier who have high intensity and prolonged contact. Analyses examining trichloroethylene exposure using either the JEM of JTEM assigned a cumulative TCE exposure index of none to low, medium high and substantial, defined as the product of exposure probability x intensity x duration with the following cutpoints: none to low, <30th percentile of cumulative exposure scores; medium, 30th–<60th percentile; high, 60th–<90th percentile; and, substantial, ≥90th percentile. The use of the German JEM identified approximately twice as many cases with any potential TCE exposure (44%) compared to the JTEM (22%) and, in both cases, few cases identified with substantial exposure, 7% by JEM and 5% by JTEM. Pesch et al. (2000a) noted "exposure indices derived from an expert rating of job tasks can have a higher agent-specificity than indices derived from job titles." For this reason, the JTEM approach with consideration of job tasks is considered a more robust exposure metric for examining TCE exposure and urothelial carcinoma due to likely reduced potential for exposure misclassification compared to TCE assignment using only job history and title.

While this case-control study includes a region in the North Rhine-Westphalia region where the Arnsberg area is also located, several other regions are included as well, where the source of the trichloroethylene and chlorinated solvent exposures are expected as much less well defined. Few cases were identified as having substantial exposure to TCE and, as a result, most subjects identified as exposed to trichloroethylene probably had minimal contact, averaging concentrations of about 10 ppm or less (NRC, 2006).

Pesch B, Haerting H, Ranft U, Klimpel A, Oelschlagel B, Schill W, and the MURC Study Group. 2000a. Occupational risk factors for urothelial carcinoma: agent-specific results from a case-control study in Germany. Int J Epidemiol 29:238−247.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, this case-control study was conducted to estimate urothelial carcinoma risk for exposure to occupational-related agents; chlorinated solvents including trichloroethylene were identified as exposures of *a priori* interest.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,035 urothelial (bladder, ureter, renal pelvis) carcinoma cases were identified from hospitals in a five-region area in Germany between 1991 and 1995. Cases were confirmed histologically. 4,298 population controls identified from local residency registries in the five-region area were frequency matched to cases by region, sex and age comprised the control series for both the urothelial carcinoma cases and the RCC cases, published as Pesch et al. (2000a). Participation rate: cases, 84%; controls, 71%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	No information in paper.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	A trained interviewer interviewed subjects using a structured questionnaire which covered occupational history and job title for all jobs held longer than one yr, medical history, and personal information. Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, trichloroethylene, and tetrachloroethylene) and chemical-specific exposure were assigned using a JTEM. Exposure index for each subject is the sum over all jobs of duration x probability x intensity. A four category grouping was used in statistical analyses defined by exposure index distribution of controls: no-low; medium, 30^{th} percentile; high, 60^{th} percentile; substantial, 90^{th} percentile.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Interviewers carried out face-to-face interview with all cases and controls. All cases were interviewed in the hospital within 6 mos of initial diagnosis. All controls had home interviews.
Blinded interviewers	No, by nature of interview location.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No, all cases and controls were alive at time of interview.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	JEM: 460 cases with TCE exposure index of medium or higher (44% exposure prevalence among cases), 71 cases with substantial exposure (7% exposure prevalence). JTEM: 157 cases with TCE exposure index of medium or higher (22% exposure prevalence among cases), and 36 males assigned substantial exposure (5% exposure prevalence). No information is presented in paper on control exposure prevalence.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, study center, and smoking.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.1.2. *Siemiatycki et al. (1994), Siemiatycki (1991).*

B.3.2.1.2.1. ***Author's abstract.***

> A population-based case-control study of the associations between various cancers and occupational exposures was carried out in Montreal, Quebec, Canada. Between 1979 and 1986, 484 persons with pathologically confirmed cases of bladder cancer and 1,879 controls with cancers at other sites were interviewed, as was a series of 533 population controls. The job histories of these subjects were evaluated by a team of chemist/hygienists for evidence of exposure to a list of 294 workplace chemicals, and information on relevant non-occupational confounders was obtained. On the basis of results of preliminary analyses and literature review, 19 occupations, 11 industries, and 23 substances were selected for in-depth multivariate analysis. Logistic regression analyses were carried out to estimate the odds ratio between each of these occupational circumstances and bladder cancer. There was weak evidence that the following substances may be risk factors for bladder cancer: natural gas combustion products, aromatic amines, cadmium compounds, photographic products, acrylic fibers, polyethylene, titanium dioxide, and chlorine. Among the substances evaluated which showed no evidence of an association were benzo(a)pyrene, leather dust, and formaldehyde. Several occupations and industries were associated with bladder cancer, including motor vehicle drivers and textile dyers.

B.3.2.1.2.2. ***Study description and comment***. Siemiatycki et al. (1994) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and bladder cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 617 newly diagnosed cases of primary bladder cancer, confirmed on the basis of histology reports, between 1979 and 1985; 484 of these participated in the study interview (78% participation). One control group (*n* = 1,295) consisted of patients with other forms of cancer (excluding lung and kidney cancer) recruited through the same study procedures and time period as the bladder cancer cases. A population-based control group (*n* = 533, 72% response), frequency matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Siemiatycki et al. (1994) presents observations of analyses examining job title, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991). Any exposure to TCE was 2% among cases (n = 8) but <1% for substantial TCE exposure (n = 5); "substantial" is defined as ≥10 years of exposure for the period up to 5 years before diagnosis. Logistic regression models adjusted for age, ethnicity, socioeconomic status, smoking, coffee consumption, and status of respondent (Siemiatycki et al., 1994) or Mantel-Henszel χ^2 stratified on age, family income, cigarette smoking, coffee, and respondent status (Siemiatycki, 1991). Odds ratios for TCE exposure are presented in Siemiatycki (1991) with 90% confidence intervals.

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of bladder cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Siemiatycki J, Dewar R, Nadon L, Gérin M. 1994. Occupational risk factors for bladder cancer: results from a case-control study in Montreal, Quebec, Canada. Am J Epidemiol 140:1061−1080.

Siemiatycki J. 1991. Risk Factors for Cancer in the Workplace. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	617 bladder cancer cases were identified among male Montreal residents between 1979 and 1985 of which 484 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung and kidney cancer cases. Participation rate: cases, 78%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O, 188 (Malignant neoplasm of bladder).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 300 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	484 cases (78% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases; Substantial TCE exposure (Exposure for $\geq$10 yrs and up to 5 yrs before disease onset), <1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, income, index for cigarette smoking, coffee, and respondent status (Siemiatycki, 1991). Age, ethnicity, socioeconomic status, smoking, coffee consumption, and status of respondent (Siemiatycki et al., 1994).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Siemiatycki et al., 1994).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.2. *Central Nervous System Cancers Case-Control Studies*

B.3.2.2.1. *De Roos et al. (2001).*

B.3.2.2.1.1. ***Author's abstract.***

> To evaluate the effects of parental occupational chemical exposures on incidence of neuroblastoma in offspring, the authors conducted a multicenter case-control study, using detailed exposure information that allowed examination of specific chemicals. Cases were 538 children aged 19 years who were newly diagnosed with confirmed neuroblastoma in 1992–1994 and were registered at any of 139 participating hospitals in the United States and Canada. One age-matched control for each of 504 cases was selected through random digit dialing. Self-reported exposures were reviewed by an industrial hygienist, and improbable exposures were reclassified. Effect estimates were calculated using unconditional logistic regression, adjusting for child's age and maternal demographic factors. Maternal exposures to most chemicals were not associated with neuroblastoma. Paternal exposures to hydrocarbons such as diesel fuel (odds ratio (OR) = 1.5; 95% confidence interval (CI): 0.8, 2.6), lacquer thinner (OR = 3.5; 95% CI: 1.6, 7.8), and turpentine (OR = 10.4; 95% CI: 2.4, 44.8) were associated with an increased incidence of neuroblastoma, as were exposures to wood dust (OR = 1.5; 95% CI: 0.8, 2.8) and solders (OR = 2.6; 95% CI: 0.9, 7.1). The detailed exposure information available in this study has provided additional clues about the role of parental occupation as a risk factor for neuroblastoma.

B.3.2.2.1.2. ***Study description and comment***. De Roos et al. (2001), a large multicenter case-control study of neuroblastoma in offspring and part of the pediatric collaborative clinical trial groups, the Children's Cancer Group and the pediatric Oncology Group, examined parental and maternal chemical exposures, focusing on solvent exposures, expanding the exposure assessment approach of Olshan et al. (1999) who examined parental occupational title among cases and controls. Neuroblastoma in patients under the age of 19 years was identified at one of 139 participating hospitals in the United States and Canada from 1992 to 1996. One population control per case s was using a telephone random digit dialing procedure and matched to the case on date of birth (+6 months for cases 3 years old or younger and +1 year for cases old than 3 years of age). A total of 741 cases and 708 controls were identified with direct interviews by telephone obtained from 538 case mothers (73% participation), 405 case fathers, 504 control mothers (71% participation), and 304 control fathers. Mothers served as proxy respondents for paternal information for 67 cases (12%) and 141 controls (28%).

A strength of the study was its use of industrial hygienist review of self-reported occupational exposure to increase specificity, reduce the number of false-positive information from self-reported exposures, and to minimize exposure misclassification bias. A parent was coded as having been exposed to individual chemicals or chemical group (halogenated hydrocarbons, paints, metals, etc.) if the industrial hygiene review determined probable exposure in any job. Individual chemicals in the halogenated hydrocarbons grouping included carbon tetrachloride, chloroform, Freon, methylene chloride, perchloroethylene and TCE. Typical of population case-control studies, reported TCE exposure was uncommon among cases and controls. Only 6 case and 8 control mothers were identified by industrial hygiene review of occupational information to have probable exposure to halogenated hydrocarbons. The few numbers prevented examination of specific chemical exposure. Of the 538 cases and 504 controls, paternal exposure to TCE was self-reported for 22 cases (5%) and 12 controls (4%) were identified with paternal TCE exposure with fewer fathers with probable TCE exposure confirmed from industrial hygiene expert review, 9 cases (2%) and 7 controls (2%).

Overall, this study has a low sensitivity and statistical power for evaluating parental TCE exposure and neuroblastoma in offspring due to the low exposure prevalence to TCE. Although study investigators took effort to reduce false positive reporting, exposure misclassification bias may still be possible from false negative reporting of occupational information. As discussed by study authors, job duty information reported by parents was best used to infer exposure to chemical categories but was not detailed sufficiently to infer specific exposures. The study's reported risk estimates for TCE exposure are imprecise and do not provide support for or against an association.

De Roos AJ, Olshan AF, Teschke K, Poole Ch, Savitz DA, Blatt J, Bondy ML, Pollock BH. 2001. Parental occupational exposure to chemicals and incidence of neuroblastoma in offspring. Am J Epidemiol 154:106−114.

Olshan AF, De Roos AJ, Teschke K, Neglin JP, Stram DO, Pollock BH, Castleberry RP. 1999. Neuroblastoma and parental occupation. Cancer Causes Control 10:539−549.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This multicenter population case-control study examined parental chemical-specific occupational exposures using detailed exposure information.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	538 cases of neuroblastoma in children <19 years of age and diagnosed between 1992 and 1994 at any of 139 United States or Canadian hospitals participating in the Children's Cancer Group and Pediatric Oncology Group studies. 504 population controls were selected through random digit dialing and matched (1:1) with cases on date of birth. Controls could not be located for 34 cases. 538 of 741 potentially eligible cases (73% participation rate). 504 of 681 potentially eligible controls (74% participation rate).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported exposure to any of 65 chemicals, compounds, or broad categories was obtained from structured questionnaire. An industrial hygienist confirmed each respondent's self-reported chemical exposure responses. Exposures were not assigned using JEM. TCE exposure examined in analysis as separate exposure and as one of several chemicals in the broader category of "halogenated hydrocarbons."

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interview with mother and father of each case and control.
Blinded interviewers	Not identified in paper.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No proxy information on maternal exposure; direct interview with mother was obtained for 537 cases and 503 controls. Analysis of paternal chemical exposures did not include information on paternal exposure from proxy interviews.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Self-reported TCE exposure: 22 cases (5% exposure prevalence) and 12 controls (4% exposure prevalence). IH-reviewed TCE exposure: 9 cases (2% exposure prevalence) and 7 controls (2% exposure prevalence).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Analyses of maternal and paternal occupational exposure each adjusted for child's age, maternal race, maternal age, and maternal education.
Statistical methods	Separate analyses are conducted for maternal and paternal exposure using logistic regression methods.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes, results are well documented.

B.3.2.2.2. *Heineman et al. (1994).*

B.3.2.2.2.1. ***Author's abstract.***

> Chlorinated aliphatic hydrocarbons (CAHs) were evaluated as potential risk factors for astrocytic brain tumors. Job-exposure matrices for six individual CAHs and for the general class of organic solvents were applied to data from a case-control study of brain cancer among white men. The matrices indicated whether the CAHs were likely to have been used in each industry and occupation by decade (1920–1980), and provided estimates of probably and intensity of exposure for "exposed" industries and occupations. Cumulative exposure indices were calculated for each subject.
>
> Associations of astrocytic brain cancer were observed with likely exposure to carbon tetrachloride, methylene chloride, tetrachloroethylene, and trichloroethylene, but were strongest for methylene chloride. Exposure to chloroform or methyl chloroform showed little indication of an association with brain cancer. Risk of astrocytic brain tumors increase with probability and average intensity of exposure, and with duration of employment in jobs considered exposed to methylene chloride, but not with a cumulative exposure score. These trends could not be explained by exposures to the other solvents.

B.3.2.2.2.2. ***Study description and comment***. Heineman et al. (1994) studied the association between astrocytic brain cancer (ICD-9 codes 191, 192, 225, and 239.7) and occupational exposure to chlorinated aliphatic hydrocarbons. Cases were identified using death certificates from southern Louisiana, northern New Jersey, and the Philadelphia area. This analysis was limited to white males who died between 1978 and 1981. Controls were randomly selected from the death certificates of white males who died of causes other than brain tumors, cerebrovascular disease, epilepsy, suicide, and homicide. The controls were frequency matched to cases by age, year of death, and study area.

Next-of-kin were successfully located for interview for 654 cases and 612 controls, which represents 88 and 83% of the identified cases and controls, respectively. Interviews were completed for 483 cases (74%) and 386 controls (63%). There were 300 cases of astrocytic brain cancer (including astrocytoma, glioblastoma, mixed glioma with astrocytic cells). The ascertainment of type of cancer was based on review of hospital records which included pathology reports for 229 cases and computerized tomography reports for 71 cases. After excluding 66 controls with a possible association between occupational exposure to chlorinated aliphatic hydrocarbons and cause of death (some types of cancer, cirrhosis of the liver), the final analytic sample consisted of 300 cases and 320 controls.

In the next-of-kin interviews, the work history included information about each job held since the case (or control) was 15 years old (job title, description of tasks, name and location of company, kinds of products, employment dates, and hours worked per week). Occupation and industry were coded based on four digit Standard Industrial Classification and Standard Occupational Classification (Department of Commerce) codes. The investigators developed matrices linked to jobs with likely exposure to six chlorinated aliphatic hydrocarbons (carbon tetrachloride, chloroform, methyl chloroform, methylene dichloride, tetrachloroethylene, and trichloroethylene), and to organic solvents (Gomez et al., 1994). This assessment was done blinded to case-control status. Exposure was defined as the probability of exposure to a substance (the highest probability score for that substance among all jobs), duration of employment in the exposed occupation and industry, specific exposure intensity categories, average intensity score (the three-level semiquantitative exposure concentration assigned to each job multiplied by duration of employment in the job, summed across all jobs), and cumulative exposure score (weighted sum of years in all exposed jobs with weights based on the square of exposure intensity [1, 2, 3] assigned to each job). Secular trends in the use of specific chemicals were considered in the assignment of exposure potential. Exposures were lagged 10 or 20 years to account for latency. Thus, this exposure assessment procedure was quite detailed.

The strengths of this case-control study include a large sample size, detailed work histories including information not just about usual or most recent industry and occupation, but also about tasks and products for all jobs held since age 15, and comprehensive exposure assessment and analysis along several different dimensions of exposure. The major limitation was the lack of direct exposure information and potential inaccuracy of the description of work histories that was obtained from next-of-kin interviews. The authors acknowledge this limitation in the report, and in response to a letter by Norman (1996) criticizing the methodology and interpretation of the study with respect to the observed association with methylene chloride, Heineman et al. (1994) noted that while the lack of direct exposure information must be interpreted cautiously, it does not invalidate the results. Differential recall bias between cases and controls was unlikely because work histories came from next-of-kin for both groups and, the industrial hygienists made their judgments blinded to disease status. Nondifferential misclassification is possible due to underreporting of job information by next of kin and would, on average, attenuate true associations.

Heineman EF, Cocco P, Gomez MR, Dosemeci M, Stewart PA, Hayes RB, Zahm SH, Thomas TL, Blair A. 1994. Occupational exposure to chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. Am J Ind Med 26:155−169.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, study further examines six specific solvents including trichloroethylene in a previous study of brain cancer which reported association with electrical equipment production and repair.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Brain cancer deaths among white males in southern Louisiana, northern New Jersey and Philadelphia, Pennsylvania, were identified using death certificates (n = 741). Controls were randomly selected (source not identified in paper) among other cause-specific deaths among white male residents of these areas and matched to cases by age, year of death and study area (n = 741). Participation rate, 483 of 741 (65% of cases with brain cancer); 386 of 741 controls (52%). Of the 483, 300 deaths were due to astrocytic brain cancer.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD, 9th revision, Codes 191, 192, 225, 239.7.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	The job-exposure-matrix of Gomez et al. (1994) was used to assign potential exposure to 6 solvents including trichloroethylene.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% Face-to-Face	Interview with next-of-kin but paper does not identify whether telephone or face-to-face.
Blinded interviewers	Interviewer was blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Proxy information was obtained from 100% of cases and controls.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	TCE exposure prevalence: 128 cases (43%) and 125 controls (39%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Stratified analysis controlled for age, year of death and study area; employment in electronics-related occupations was included in addition in logistic regression analyses.
Statistical methods	Stratified analysis using 2 × 2 tables and logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.3. *Colon and Rectal Cancers Case-Control Studies*

B.3.2.3.1. *Goldberg et al. (2001), Simiatycki (1991).*

B.3.2.3.1.1. ***Author's abstract.***

> BACKGROUND: We conducted a population-based case-control study in Montreal, Canada, to explore associations between hundreds of occupational circumstances and several cancer sites, including colon. METHODS: We interviewed 497 male patients with a pathologically confirmed diagnosis of colon cancer, 1514 controls with cancers at other sites, and 533 population-based controls. Detailed job histories and relevant potential confounding variables were obtained, and the job histories were translated by a team of chemists and industrial hygienists into a history of occupational exposures. RESULTS: We found that there was reasonable evidence of associations for men employed in nine industry groups (adjusted odds ranging from 1.1 to 1.6 per a 10-year increase in duration of employment), and in 12 job groups (OR varying from 1.1 to 1.7). In addition, we found evidence of increased risks by increasing level of exposures to 21 occupational agents, including polystyrene (OR for "substantial" exposure (OR(subst) = 10.7), polyurethanes (OR(subst) = 8.4), coke dust (OR(subst) = 5.6), mineral oils (OR(subst) = 3.3), polyacrylates (OR(subst) = 2.8), cellulose nitrate (OR(subst) = 2.6), alkyds (OR(subst) = 2.5), inorganic insulation dust (OR(subst) = 2.3), plastic dusts (OR(subst) = 2.3), asbestos (OR(subst) = 2.1), mineral wool fibers (OR(subst) = 2.1), glass fibers (OR(subst) = 2.0), iron oxides (OR(subst) = 1.9), aliphatic ketones (OR(subst) = 1.9), benzene (OR(subst) = 1.9), xylene (OR(subst) = 1.9), inorganic acid solutions (OR(subst) = 1.8), waxes, polishes (OR(subst) = 1.8), mononuclear aromatic hydrocarbons (OR(subst) = 1.6), toluene (OR(subst) = 1.6), and diesel engine emissions (OR(subst) = 1.5). Not all of these effects are independent because some exposures occurred contemporaneously with others or because they referred to a group of substances. CONCLUSIONS: We have uncovered a number of occupational associations with colon cancer. For most of these agents, there are no published data to support or refute our observations. As there are few accepted risk factors for colon cancer, we suggest that new occupational and toxicologic studies be undertaken focusing on the more prevalent substances reported herein.

B.3.2.3.1.2. ***Study description and comment***. Goldberg et al. (2001) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and colon cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 607 newly diagnosed cases of primary colon cancer (ICD9, 153), confirmed on the basis of histology reports, between 1979 and 1985; 497 of these participated in the study interview (81.9% participation). One control group (*n* = 1,514) consisted of patients with other forms of cancer (excluding cancers of the lung, peritoneum, esophagus, stomach, small intestine, rectum, liver and intrahepatic bile ducts, gallbladder and extrahepatic bile ducts and pancreas) recruited through the same study procedures and time period as the colon cancer cases. A population-based control group (n = 533, 72% response), frequency matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Goldberg et al. (2001) presents observations of analyses examining industries, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991). Any exposure to TCE was 2% among cases (n = 12) and 1% for substantial TCE exposure (n = 7); "substantial" is defined as $\geq$10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for a number of nonoccupational variables including age, ethnicity, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source and cooking source in childhood home, consumption of nonpublic water supply, and body mass index (Goldberg et al., 2001) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991). Odds ratios for TCE exposure are presented in Siemiatycki (1991) with 90% confidence intervals.

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of colon cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Goldberg MS, Parent M-E, Siemiatycki J, Desy M, Nadon L, Richardson L, Lakhani R, Lateille B, Valois M-F. 2001. A case-control study of the relationship between the risk of colon cancer in men and exposure to occupational agents. Am J Ind Med 39:5310−546.

Siemiatycki J. 1991. Risk Factors for Cancer in the Workplace. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	607 colon cancer cases were identified among male Montreal residents between 1979 and 1985 of which 497 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung peritoneum and other digestive cancers. Participation rate: cases, 81.9%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-9, 153 (Malignant neoplasm of colon).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	497 cases (81.9% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases; Substantial TCE exposure (Exposure for ≥10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, ethnicity, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source and cooking source in childhood home, consumption of nonpublic water supply, and body mass index (Goldberg et al., 2001). Age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Goldberg et al., 2001).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.3.2. *Dumas et al. (2000), Siemiatycki (1991).*

B.3.2.3.2.1. *Author's abstract.*

> In 1979, a hypothesis-generating, population-based case-control study was undertaken in Montreal, Canada, to explore the association between occupational exposure to 294 substances, 130 occupations and industries, and various cancers. Interviews were carried out with 3,630 histologically confirmed cancer cases, of whom 257 had rectal cancer, and with 533 population controls, to obtain detailed job history and data on potential confounders. The job history of each subject was evaluated by a team of chemists and hygienists and translated into occupational exposures. Logistic regression analyses adjusted for age, education, cigarette smoking, beer consumption, body mass index, and respondent status were performed using population controls and cancer controls, e.g., 1,295 subjects with cancers at sites other than the rectum, lung, colon, rectosigmoid junction, small intestine, and peritoneum. We present here the results based on cancer controls. The following substances showed some association with rectal cancer: rubber dust, rubber pyrolysis products, cotton dust, wool fibers, rayon fibers, a group of solvents (carbon tetrachloride, methylene chloride, trichloroethylene, acetone, aliphatic ketones, aliphatic esters, toluene, styrene), polychloroprene, glass fibers, formaldehyde, extenders, and ionizing radiation. The independent effect of many of these substances could not be disentangled as many were highly correlated with each other.

B.3.2.3.2.2. *Study description and comment*. Dumas et al. (2000) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and rectal cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 304 newly diagnosed cases of primary rectal cancers, confirmed on the basis of histology reports, between 1979 and 1985; 257 of these participated in the study interview (84.5% response). One control group (*n* = 1,295) consisted of patients with other forms of cancer (excluding lung cancer and other intestinal cancers) recruited through the same study procedures and time period as the rectal cancer cases. A population-based control group (*n* = 533), frequency matched by age strata, was drawn using electoral lists and random digit dialing (72% response). The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews. The percentage of proxy respondents was 15.2% for cases, 19.7% for other cancer controls, and 12.6% for the population controls.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Any exposure to TCE was 5% among cases (*n* = 12) and 1% for substantial TCE exposure (*n* = 3); “substantial” is defined as $\geq$10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, education, respondent status, cigarette smoking, beer consumption and body mass index (Dumas et al., 2000) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991). Dumas et al. (2000) presents observations of analyses examining industries, occupation, and some chemical-specific exposures, including TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). Odds ratios for TCE exposure are presented in Siemiatycki (1991) with 90% confidence intervals and 95% confidence intervals in Dumas et al. (2000).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of rectal cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Dumas S, Parent M-E, Siemiatycki J, Brisson J. 2000. Rectal cancer and occupational risk factors: a hypothesis-generating, exposure-based case-control study. Int J Cancer 87:874−879.

Siemitycki J. 1991. Risk Factors for Cancer in the Workplace. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	304 rectal cancer cases were identified among male Montreal residents between 1979 and 1985 of which 294 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung and other intestinal cancer cases. Participation rate: cases, 84.5%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O, 154 (Malignant neoplasm of rectum, rectosigmoid junction and anus).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face to face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	294 cases (78% response), 533 population controls (72% response). Exposure prevalence: Any TCE exposure, 5% cases; Substantial TCE exposure (Exposure for ≥10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, education, respondent status, cigarette smoking, beer consumption and body mass index (Dumas et al., 2000). Age, family income, cigarette smoking, coffee, ethnic origin, and beer consumption (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Dumas et al., 2000).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.3.3. *Fredriksson et al. (1989).*

B.3.2.3.3.1. ***Author's abstract.***

> A case-control study on colon cancer was conducted encompassing 329 cases and 658 controls. Occupations and various exposures were assessed by questionnaires. A decreased risk was found in persons with physically active occupations. This effect was most pronounced in colon descendens and sigmoideum with an odds ratio (OR) of 0.49 whereas no reduced risk was found for right-sided colon cancer. Regarding specific jobs, reduced ORs were found for agricultural, forestry, and saw mill workers and increased OR for railway employees. High-grade exposure to asbestos or to organic solvents gave a two-fold increased risk. Regarding exposure to trichloroethylene in general, a slightly increased risk was found whereas such exposure among dry cleaners gave a 7-fold increase of the risk.

B.3.2.3.3.2. *Study description and comment*. Fredriksson et al. (1989) reported data from a population case-control study of occupational and nonoccupational exposures and rectal cancer conducted in Ureå, Sweden. The investigators identified 329 diagnosed cases of rectal cancers (ICD 8, 153), between 1980 and 1983, confirmed on the basis of histology reports and alive at the time of data collect between 1984 and 1986; 302 (165 males and 165 females) of these participated in the study interview (92% response). A population-based control group (n = 658), matched by a 1:2 ratio to cases on age sex and county residence, was drawn using the Swedish National Population Register list; 623 (306 males and 317 females) returned mailed questionnaires and participated in the study (95% response).

The occupational assessment consisted of a detailed description of each job held during the working lifetime, including details on specific occupations and exposures. Occupation information was provided directly from each case and control given the study's eligibility requirement of being alive at the time of data collection. A team of experts independently classified three exposures of interest (asbestos, organic solvents, and impregnating agents) into two categories, low grade exposure and high grade exposure and other chemical-specific exposures, including TCE, as either "exposed" or "unexposed." Fredriksson et al. (1989) do not define these categories nor do they provide information on exposure potential, frequency of exposure, or concentration of exposure. No information is provided whether experts were blinded as to disease status.

Statistical analysis examining occupation and agent-specific exposures was carried out using Mantel-Haenszel χ^2 stratified on age, sex, and an index of physical activity. Odds ratios associated with specific chemical exposure are presented with their 95% confidence intervals.

The strengths of this study were its specific information about job duties for all jobs held and a definitive diagnosis of rectal cancer. However, the study's assignment of exposure potential from information using mailed questionnaires is considered inferior to information obtained directly from trained interviewers and expert assessment because of greater uncertainty and misclassification (Fritschi et al., 1996). The degree of potential exposure misclassification bias in this population case-control study of colon cancer is not known. Furthermore, exposure prevalence to TCE appears low, as judged by the wide confidence interval around the odds ratio. This study is considered as having decreased sensitivity for examining colon cancer and TCE given the apparent lower exposure prevalence and likely exposure misclassification bias associated with mailed questionnaire information.

Fredriksson M, Bengtsson N-O, Hardell L, Axelson O. 1989. Colon cancer, physical activity, and occupational exposure. A case-control study. Cancer 63:1838−1842.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Abstract—to evaluate occupational and nonoccupational exposures as risk factors for colon cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	302 (165 males and 165 females) cases participated in study out of 329 eligible cases reported to the Swedish Cancer Registry between 1980 and 1983, among resident of Umeå, Sweden, alive at time of data collection 1984 and 1986, and with histological-confirmed diagnosis of colon cancer. 623 (306 males and 317 females) identified from Swedish Population Registry and matched for age, sex, and county of residence. Participation rate: cases, 92%; population controls, 95%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-8, 153 (Malignant neoplasm of large intestine, except rectum).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported information on occupational exposure as obtained from a mailed questionnaire to study participants. Questionnaire sought information on complete working history, other exposures, and dietary habits. Procedure for assigning chemical exposures from job title information not described in paper.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Mailed questionnaire.
Blinded interviewers	No information in published paper.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No proxy respondents, all cases and controls alive at time of data collection.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	302 cases (92% response), 623 population controls (95% response). Exposure prevalence not calculated, published paper lacks number of TCE exposed cases and controls.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Yes, age, sex, and index of physical activity.
Statistical methods	Mantel-Haenszel.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.4. *Esophageal Cancer Case-Control Studies*

B.3.2.4.1. *Parent et al. (2000a), Siemiatycki (1991).*

B.3.2.4.1.1. ***Parent et al. (2000a) abstract.***

> OBJECTIVES: To describe the relation between oesophageal cancer and many occupational circumstances with data from a population based case-control study. METHODS: Cases were 99 histologically confirmed incident cases of cancer of the oesophagus, 63 of which were squamous cell carcinomas. Various control groups were available; for the present analysis a group was used that comprised 533 population controls and 533 patients with other types of cancer. Detailed job histories were elicited from all subjects and were translated by a team of chemists and hygienists for evidence of exposure to 294 occupational agents. Based on preliminary results and a review of literature, a set of 35 occupational agents and 19 occupations and industry titles were selected for this analysis. Logistic regression analyses were adjusted for age, birthplace, education, respondent (self or proxy), smoking, alcohol, and beta-carotene intake. RESULTS: Sulphuric acid and carbon black showed the strongest evidence of an association with oesophageal cancer, particularly squamous cell carcinoma. Other substances showed excess risks, but the evidence was more equivocal-namely chrysotile asbestos, alumina, mineral spirits, toluene, synthetic adhesives, other paints and varnishes, iron compounds, and mild steel dust. There was considerable overlap in occupational exposure patterns and results for some of these substances may be mutually confounded. None of the occupations or industry titles showed a clear excess risk; the strongest hints were for warehouse workers, food services workers, and workers from the miscellaneous food industry. CONCLUSIONS: The data provide some support for an association between oesophageal cancer and a handful of occupational exposures, particularly sulphuric acid and carbon black. Many of the associations found have never been examined before and warrant further investigation.

B.3.2.4.1.2. ***Study description and comment***. Parent et al. (2000a) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and esophageal cancer conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 129 newly diagnosed cases of primary esophageal cancers, confirmed on the basis of histology reports, between 1979 and 1985; 99 of these participated in the study interview (76.7% response). One control group consisted of patients with other forms of cancer recruited through the same study procedures and time period as the esophageal cancer cases. A population-based control group (n = 533), frequency matched by age strata, was drawn using electoral lists and random digit dialing (72% response). Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents.

The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews. A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Any exposure to TCE was 1% among cases ($n = 1$) and 1% for substantial TCE exposure ($n = 1$); "substantial" is defined as $\geq$10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, education, respondent status, birthplace, cigarette smoking, beer consumption spirits consumption and beta-carotene intake (Parent et al., 2000a) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, and an index for alcohol consumption (Siemiatycki, 1991). Parent et al. (2000a) presents observations of analyses examining industries, occupation, and some chemical-specific exposures, including solvents, but not TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). Odds ratios for TCE exposure are presented in Siemiatycki (1991) with 90% confidence intervals and 95% confidence intervals in Parent et al. (2000a).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of esophageal cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Parent M-E, Siemiatycki J, Fritschi L. 2000a. Workplace exposures and oesophageal cancer. Occup Environ Med 57:325−334.

Siemitycki J. 1991. Risk Factors for Cancer in the Workplace. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	129 esophageal cancer cases were identified among male Montreal residents between 1979 and 1985 of which 99 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of all other cancer controls. Participation rate: cases, 76.7%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O, 150 (Malignant neoplasm of esophagus).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	99 cases (76.7% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 1% cases; Substantial TCE exposure (Exposure for $\geq$10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, education, respondent status, birthplace, cigarette smoking, beer consumption spirits consumption and beta-carotene intake (Parent et al., 2000a). Age, family income, cigarette smoking, and index for alcohol consumption (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Parent et al., 2000a).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.5. *Liver Cancer Case-Control Studies*

B.3.2.5.1. *Lee et al. (2003).*

B.3.2.5.1.1. ***Author's abstract.***

> Aims: To investigate the association between cancer mortality risk and exposure to chlorinated hydrocarbons in groundwater of a downstream community near a contaminated site. Methods: Death certificates inclusive for the years 1966–97 were collected from two villages in the vicinity of an electronics factory operated between 1970 and 1992. These two villages were classified into the downstream (exposed) village and the upstream (unexposed) according to groundwater flow direction. Exposure classification was validated by the contaminant levels in 49 residential wells measured with gas chromatography/mass spectrometry. Mortality odds ratios (MORs) for cancer were calculated with cardiovascular-cerebrovascular diseases as the reference diseases. Multiple logistic regressions were performed to estimate the effects of exposure and period after adjustment for age. Results: Increased MORs were observed among males for all cancer, and liver cancer for the periods after 10 years of latency, namely, 1980–89, and 1990–97. Adjusted MOR for male liver cancer was 2.57 (95% confidence interval 1.21 to 5.46) with a significant linear trend for the period effect. Conclusion: The results suggest a link between exposure to chlorinated hydrocarbons and male liver cancer risk. However, the conclusion is limited by lack of individual information on groundwater exposure and potential confounding factors.

B.3.2.5.1.2. ***Study description and comment***. Exposure potential to chlorinated hydrocarbons was assigned in this community case-control study of liver cancer in males >30 years of age using residency as coded on death certificates obtained from local household registration offices. No information is available to assess the completeness of death reporting to the local registration office. Of the 1,333 deaths between 1966 and 1997 in two villages surrounding a hazardous waste site, an electronics factory operating between 1970 and 1992 in Taoyuan, Taiwan,[1] 266 cancer deaths were identified; 53 liver cancer deaths, 39 stomach cancer deaths, 26 colorectal deaths, and 41 lung cancer deaths. Controls were identified from 344 deaths due to cardiovascular and cerebrovascular diseases, without arrhythmia; 286 were included in the statistical analysis. Residents from a village north and northeast of the plant were considered exposed and residents living south considered unexposed to chlorinated hydrocarbons. Statistical analyses are limited to Mantel-Haenszel chi-square approaches stratified by sex and age and, for male cases and controls, logistic regression with age as a covariate. Socioeconomic characteristics were similar between residents of the two villages (Wang, 2004). The study does

[1] The factory's workers were subjects in the cohort studies of Chang et al. (2003, 2005) and Sung et al. (2007, 2008).

not include control for potential confounding from hepatitis virus; high rates of hepatitis B and C are endemic to Taiwan and northern Taiwan, the location of this study, has a high prevalence of hepatitis C virus infection (Lee et al., 2003). Confounding would be introduced if the prevalence of hepatitis C differed between the two villages.

Exposure assessment is quite limited and misclassification bias likely high using residence address as recorded on the death certificate as a surrogate for consumption of contaminated drinking water. The paper not only lacks information on intensity and duration of hydrocarbon exposures to individual cases and controls, but no information is available on an estimate of the amount of TCE ingested. Information on residence length, population mobility, and chemical usage at the plant are lacking. Similarly, well water monitoring is sparse, based on seven chlorinated hydrocarbons monitored over a 7 month period between 1999−2000 in 69 groundwater samples from 44 wells to the north and northeast, or downstream from the factory, and in 5 groundwater samples from 2 wells to the south or upstream from the factory. Monitoring from other time periods is lacking with no information available to judge if current monitoring are representative of past concentrations. Median concentrations (μg/L or ppb) and ranges (μg/L or ppb) for these seven chemicals are below. Highest concentration of contaminants was from wells closest to the factory boundary with concentrations detected at or close to maximum contaminant levels in wells located 0.5 mile (1,000 meters) away. A municipal system supplied water to upstream village residents (start date no identified); however, wells served as source for water to of the north or downstream village residents. The exposure assessment does not consider potential occupational exposure.

Chemical	**Downstream**		**Upstream**	
	Median	**Range**	**Median**	**Range**
Trichloroethylene	28	N.D.−1,791	0.1	0.1−0.1
Perchloroethylene	3	N.D.−5,228	0.05	N.D.−0.1
cis-1,2-dichloroethylene	3	N.D.−1,376	N.D.	N.D.
1,1-dichloroethane	2	N.D.−228	0.05	N.D.−0.1
1,1-dichloroethylene	1	N.D.−1,240	N.D.	N.D.
Vinyl chloride	0.003	N.D.−72	N.D.	N.D.

N.D. = not detected

Lee L J-H, Chung C-W, Ma Y-C, Wang G-S, Chen P-C, Hwang Y-H, Wang J-D. 2003. Increased mortality odds ratio of male liver cancer in a community contaminated by chlorinated hydrocarbons in groundwater. Occup Environ Med 60:364−369.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Study hypothesis of investigating cancer mortality risk and exposure to chlorinated hydrocarbons in groundwater.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Deaths in 1966−1997 identified from local housing registration offices among residents in two villages were the source for case and control series. The two villages were north (contaminated community) and south (unexposed) of an electronics factory declared as a hazardous waste site. No information if all death among residents were reported to registration office. Cases: 53 liver cancer deaths in males and females, 51 included in statistical analysis (96%); stomach cancer deaths (*n* = 39), colon and rectum deaths (*n* = 26), and lung cancer deaths (*n* = 41). Paper does not present numbers of stomach, colo-rectal and lung cancer deaths used in statistical analyses. Controls: 344 cardiovascular-cerebrovascular CV-CB disease deaths, 286 CV-CB deaths without arrhythmia included in statistical analysis (83%).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-9.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Exposure potential to chlorinated hydrocarbons in drinking water was inferred from residence address on deaths certificate.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	NA, Record based information.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	NA
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Liver cancer case exposure prevalence [downstream village resident], 53% (*n* = 24 males, *n* = 4 females). Control exposure prevalence [upstream village resident], 30% (*n* = 44 males, *n* = 41 females).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Sex and age (categorical). No control for potential confounding due to hepatitis virus (for liver cancer) or smoking (for lung cancer analyses).
Statistical methods	Mantel-Haenszel Chi square. Multiple logistic regressions (males deaths only).
Exposure-response analysis presented in published paper	No, MORs presented by time period.
Documentation of results	Inadequate, the paper does not discuss mobility patterns of residents, percentage of population who may have moved from area, pr completeness of death ascertainment using certificates obtained from local housing registration offices.

MOR = mortality odds ratio.

B.3.2.6. *Lymphoma Case-Control Studies*

B.3.2.6.1. *Wang et al. (2008).*

B.3.2.6.1.1. ***Author's abstract.***

> A population-based case-control study involving 601 incident cases of non-Hodgkin lymphoma (NHL) and 717 controls was conducted in 1996-2000 among Connecticut women to examine associations with exposure to organic solvents. A job-exposure matrix was used to assess occupational exposures. Increased risk of NHL was associated with occupational exposure to chlorinated solvents (odds ratio (OR) = 1.4, 95% confidence interval (CI): 1.1, 1.8) and carbon tetrachloride (OR = 2.3, 95% CI: 1.3, 4.0). Those ever exposed to any organic solvent in work settings had a borderline increased risk of NHL (OR = 1.3, 95% CI: 1.0, 1.6); moreover, a significantly increased risk was observed for those with average probability of exposure to any organic solvent at medium-high level (OR = 1.5, 95% CI: 1.1, 1.9). A borderline increased risk was also found for ever exposure to formaldehyde (OR = 1.3, 95% CI: 1.0, 1.7) in work settings. Risk of NHL increased with increasing average intensity ($P = 0.01$), average probability ($p< 0.01$), cumulative intensity ($P = 0.01$), and cumulative probability ($p < 0.01$) level of organic solvent and with average probability level ($P = 0.02$) and cumulative intensity level of chlorinated solvent ($P = 0.02$). Analyses by NHL subtype showed a risk pattern for diffuse large B-cell lymphoma similar to that for overall NHL, with stronger evidence of an association with benzene exposure. Results suggest an increased risk of NHL associated with occupational exposure to organic solvents for women.

B.3.2.6.1.2. ***Study description and comment***. This population case-control study of non-Hodgkin's lymphoma in Connecticut women was designed to examine possible personal and occupational risk factors for NHL. The publication of Wang et al. (2008) examined solvent exposure and adopted a job-exposure matrix to assign exposure potential to nine chemicals—benzene, formaldehyde, chlorinated solvents, chloroform, carbon tetrachloride, dichloromethane, methyl chloride and trichloroethylene. Histologically-confirmed incident cases of NHL in women aged between 21 and 84 years of age and diagnosed in Connecticut between 1996 and 2000 were identified from the Connecticut Cancer Registry, a SEER reporting site, with population controls having Connecticut address identified from random digit dialing for women <65 years of age, or by random selection from Centers for Medicare and Medicaid Service files for women aged 65 year or older. Controls were frequency matched to cases within 5-year age groups. Face-to-face interviews were completed for 601 (72%) cases and 717 controls (69% of those identified from random digit dialing and 47% identified using Health Care Financing Administration files).

Trained interviewers administered a structured questionnaire through in-person interviews with cases and controls to collect information on diet, nutrition, and alcohol intake; reproductive factors; hair dye use; and lifetime occupational history of all jobs held $\geq$1 year. Jobs were coded to standardized occupational classification and standardized industry classification titles and assigned probability and intensity of exposure to formaldehyde and nine other solvents (benzene, any chlorinated solvents, dichloroethylene, chloroform, methylene chloride, dichloroethane, methyl chloride, TCE and carbon tetrachloride) using a job-exposure matrix developed by the National Cancer Institutes (Gomez et al., 1994; Dosemeci et al., 1994). All jobs held up to a year before cancer diagnosis were assigned blinded as to disease status potential exposure to each exposure of interest. Lifetime exposure potential for cases and controls was based on exposure duration and a weighted score for exposure intensity and probability of each occupational and industry and defined as a cumulative exposure metric, average metric, or ever/never metric. Of the 601 cases, 77 (13%) were assigned with potential TCE exposure over their lifetime; eight cases were assigned potential for high intensity exposure, but with low probability and the 31 cases identified with medium and high probability of exposure were considered as having low intensity exposure potential. The low exposure prevalence to TCE, overall, and few subjects identified with confidence with high TCE exposure intensity or probability implies exposure misclassification bias is likely, and likely nondifferential, notably for high exposure categories (Dosemeci et al., 1990).

Association between NHL and individual occupational solvent exposure was assessed using unconditional logistic regression model which adjusted for age, family history of hematopoietic cancer, alcohol consumption and race. Statistical analyses treated exposure defined as a categorical variable, divided into tertiles based on the distribution of controls, in logistic regression analyses and as a continuous variable, whenever possible, to test for linear trend. Polytomous logistic regress was used to evaluate the association between histologic subtypes of NHL (DLBCL, follicular lymphoma, or chronic lymphocytic leukemia/small lymphocytic lymphoma) and exposure. The largest number of cases was of the cell type DLBCL.

Strength of this study is assignment of TCE exposure potential to individual subjects using a validated job-exposure matrix, although uncertainty accompanied exposure assignment and TCE exposure was largely of low intensity/low probability, and no cases with medium to high intensity/probability. Resultant misclassification bias would dampen observed associations for high exposure potential categories. Low prevalence of high intensity TCE exposure would reduce the study's statistical power.

Wang R, Zhang Y, Lan Q, Holford TR, Leaderer B, Zahm SH, Boyle P, Dosemeci M, Rothman N, Zhu Y, Qin Q, Zheng T. 2009. Occupational exposure to solvents and risk of non-Hodgkin lymphoma in Connecticut women.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study evaluated multiple potential risk factors of NHL in a population-based case-control study of Connecticut women. Occupational exposure to TCE was not an *a priori* hypothesis.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	601 (832 eligible) cases of NHL, diagnosed between 1996 and 2000 among women, age 20 to 84 yrs and residents of Connecticut and histologically-confirmed, were identified from the Yale Comprehensive Cancer Center's Rapid Case Ascertainment Shared Resource, a component of the Connecticut Tumor Registry; 717 (number of eligible controls not identified) population controls were randomly identified using random digit dialing, if age <65 yrs, or from Medicare and Medicaid Service files, for women aged 65 yrs or older and stratified by sex and 5-yr age groups.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL and chronic lymphatic leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O-2 [Codes, M-9590-9642, 9690-9701, 9740-9750].
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	All jobs held for >1 yr were assigned to standardized occupation and industry classifications. Using job exposure matrix of NCI (Gomez et al., 1994; Dosemeci et al., 1994), probability of exposure level (low, medium and high) and intensity (very low, low, medium and high) to TCE and other solvents (benzene, any chlorinated solvents, dichloroethylene, chloroform, methylene chloride, dichloroethane, methyl chloride, carbon tetrachloride, and formaldehyde) was assigned blinded as to case or control status.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Face-to-face interview with questionnaire for detailed information about medical history, lifestyle factors, education, lifetime occupational history (all jobs held >1 yr).
Blinded interviewers	Unblinded interviews.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	None.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	601 cases (72% participation) and 717 controls (69% participation for random digit dialing controls and 47% participation for HCFA controls). Exposure prevalence, ever exposed to TCE, 77 (13%) NHL cases; medium to high TCE intensity, 13 NHL cases (2%); medium to high TCE probability, 34 cases (6%). All 34 cases with medium to high TCE probability assigned low intensity exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, family history of hematopoietic cancer, alcohol consumption and race.
Statistical methods	Unconditional logistic regression.
Exposure-response analysis presented in published paper	Yes, by exposure intensity and by exposure probability.
Documentation of results	Yes.

B.3.2.6.2. *Costantini et al. (2008), Miligi et al. (2006).*

B.3.2.6.2.1. ***Costantini et al. (2008) abstract.***

> **Background** While there is a general consensus about the ability of benzene to induce acute myeloid leukemia (AML), its effects on chronic lymphoid leukemia and multiple myeloma (MM) are still under debate. We conducted a population-based case–control study to evaluate the association between exposure to organic solvents and risk of myeloid and lymphoid leukemia and MM.
> **Methods** Five hundred eighty-six cases of leukemia (and 1,278 population controls), 263 cases of MM (and 1,100 population controls) were collected. Experts assessed exposure at individual level to a range of chemicals.
> **Results** We found no association between exposure to any solvent and AML. There were elevated point estimates for the associations between medium/high benzene exposure and chronic lymphatic leukemia (OR: 1.8, 95% CI¼0.9–3.9) and MM (OR: 1.9, 95% CI: 0.9–3.9). Risks of chronic lymphatic leukemia were somewhat elevated, albeit with wide confidence intervals, from medium/high exposure to xylene and toluene as well.
> **Conclusions** We did not confirm the known association between benzene and AML, though this is likely explained by the strict regulation of benzene in Italy nearly three decades prior to study initiation. Our results support the association between benzene, xylene, and toluene and chronic lymphatic leukemia and between benzene and MM with longer latencies than have been observed for AML in other studies.

B.3.2.6.2.2. ***Miligi et al. (2006) abstract.***

> BACKGROUND: A number of studies have shown possible associations between occupational exposures, particularly solvents, and lymphomas. The present investigation aimed to evaluate the association between exposure to solvents and lymphomas (Hodgkin and non-Hodgkin) in a large population-based, multicenter, case-control study in Italy. METHODS: All newly diagnosed cases of malignant lymphoma in men and women age 20 to 74 years in 1991-1993 were identified in 8 areas in Italy. The control group was formed by a random sample of the general population in the areas under study stratified by sex and 5-year age groups. We interviewed 1428 non-Hodgkin lymphoma cases, 304 Hodgkin disease cases, and 1530 controls. Experts examined the questionnaire data and assessed a level of probability and intensity of exposure to a range of chemicals. RESULTS: Those in the medium/high level of exposure had an increased risk of non-Hodgkin lymphoma with exposure to toluene (odds ratio = 1.8; 95% confidence interval = 1.1-2.8), xylene 1.7 (1.0-2.6), and benzene 1.6 (1.0-2.4). Subjects exposed to all 3 aromatic hydrocarbons (benzene, toluene, and xylene; medium/high intensity compared with none) had an odds ratio of 2.1 (1.1-4.3). We observed an increased risk for Hodgkin disease for those exposed to technical solvents (2.7; 1.2-6.5) and aliphatic solvents (2.7; 1.2-5.7). CONCLUSION: This study suggests that

aromatic and chlorinated hydrocarbons are a risk factor for non-Hodgkin lymphomas, and provides preliminary evidence for an association between solvents and Hodgkin disease.

B.3.2.6.2.3. ***Study description and comment***. This series of papers of a population case-control study of lymphomas in 11 areas in Italy (Costantini et al., 2001) and occupation examines author's assigned exposure to TCE and other solvents using job-specific or industry-specific questionnaires and expert rating to cases and controls. Miligi et al. (2006) reported findings for non-Hodgkin lymphoma, a category which included chronic lymphocytic leukemia, NHL subtypes, and Hodgkin lymphoma in 8 regions and Constantini et al. (2008) presented observations for specific leukemia subtypes and multiple myeloma in 7 regions (8 regions for chronic lymphocytic leukemia). Exclusion of the regions in the original study does not appear to greatly reduce study power or to introduce a selection bias. For example, Miligi et al. (2006) included 1,428 of the 1,450 total NHL cases, the largest percentage of all lymphoma subtypes. The number of other lymphoma subtypes was much smaller compared to NHL; 304 cases of Hodgkin disease, 586 cases of leukemia, and 263 cases of multiple myeloma. All cases were identified from participating study centers and controls were randomly selected from the each area's population using stratified sampling for sex and age.

A face-to-face unblinded interview was conducted primarily at the interviewee's home with a high proportion of proxy responses among cases (19%) but not controls (5%). Bias is likely introduced by the lack of blinding of interviewers and from the high proportion of proxy interviews. A questionnaire was used to obtain information on medical history, lifestyle factors, occupational exposure and nonoccupational solvent exposures. Industrial hygiene professionals assessed the probability and intensity of exposure to individual and classes of solvents using information provided by questionnaire. Probability was classified into 3 levels (low, medium, and high) with a 4-category scale for intensity (very low, low, medium, and high). These qualitative scales lacked information on exposure concentrations and likely introduces misclassification bias that can either dampen or inflate observed risks given the study's use of multiple exposure groupings. "Very low level" was used for subjects with occupational exposure intensities judged to be comparable to the upper end of the normal range for the general population; "low-level intensity" when workplace exposure was judged to be low because of control measures but higher than background; "medium exposure" for occupational environments with moderate or poor control measures; and "high exposure" for workplaces lacking any control measures. Groupings of "very low/low" and "medium/high" exposure was used to examine association with NHL. Prevalence of medium to high TCE exposure among NHL cases was low, 3% for NHL cases and 2% for all leukemia subtypes. Whether temporal changes in TCE exposure concentrations were considered in assigning level and intensity is not known. Overall, this study has low sensitivity for examining TCE and lymphoma given the low prevalence of exposure, particularly to medium to high TCE intensity, the high proportion of proxy interviews among cases, particularly NHL cases (15%), and qualitative exposure assessment approach.

Costantini AS, Benvenuti A, Vineis P, Kriebel D, Tumino R, Ramazzotti V, Rodella S, et al., 2008. Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: evidence from the Italian multicenter case-control study. Am J Ind Med 51:803−811.

Miligi L, Costantini AS, Benvenuti A, Kreibel D, Bolejack V, et al. 2006. Occupational exposure to solvents and the risk of lymphomas. Epidemiol 17:552−561.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study evaluated TCE and other solvent exposures and lymphoma in a large population-based, multicenter, case-control study.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,732 (2,066 eligible) cases of NHL, chronic lymphatic leukemia, and Hodgkin lymphoma, diagnosed between 1991 and 1993 among men and women, age 20 to 74 yrs and residents of 8 regions in Italy, were identified from; 1,530 (2,086 eligible) population controls were randomly selected from demographic files or from sampling of National Health Service files and stratified by sex and 5-yr age groups. 586 leukemia and 263 multiple myeloma among men and women, age 20 to 74 in the period 1991−1993, from 7 regions (8 regions for chronic lymphocytic leukemia) in Italy, were identified from hospital or pathology department records or a regional cancer registry; and 1,100 population controls selected from demographic files or from sampling of National Health Service files and stratified by sex and 5-yr age groups.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL and Hodgkin’s lymphoma incidence (Miligi et al., 2006). Leukemia and multiple myeloma (Costantini et al., 2008).
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin’s lymphoma	All NHL cases were defined following NCI Working Formulation Workgroup classification and Hodgkin lymphomas defined following the Rye classification. NHL diagnosis confirmed for 334 of 1,428 cases (23%).

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	IH experts from each region using information collected on questionnaires assigned the probability of exposure level (low, medium and high) and intensity (very low, low, medium and high) to TCE and other solvents. Exposure was assigned blinded as to case or control status.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Face-to-face interview with questionnaire for detailed information about medical history, lifestyle factors, education, occupational history (period is not identified in published paper), and nonoccupational exposures including solvent exposure.
Blinded interviewers	Unblinded interviews.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	19% of all lymphoma cases and 5% of controls were with proxy respondents (Costantini et al., 2001).
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,732 cases (83% participation) and 1,530 controls (73% participation) (Miligi et al., 2006); no information on participation rate for leukemia or multiple myeloma cases or their controls in Costantini et al. (2008). Exposure prevalence, medium to high TCE intensity, 35 NHL cases (3%) (Miligi et al., 2006); 11 leukemia cases (2%) and 5 multiple myeloma cases (2%) (Costantini et al., 2008).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, region, education, and region.
Statistical methods	Multiple logistic regressions.

Exposure-response analysis presented in published paper	Yes, by exposure intensity and by duration (years) of exposure.
Documentation of results	Yes.

B.3.2.6.3. *Seidler et al. (2007).*

B.3.2.6.3.1. ***Author's abstract.***

> AIMS: To analyze the relationship between exposure to chlorinated and aromatic organic solvents and malignant lymphoma in a multi-centre, population-based case-control study. METHODS: Male and female patients with malignant lymphoma (n = 710) between 18 and 80 years of age were prospectively recruited in six study regions in Germany (Ludwigshafen/Upper Palatinate, Heidelberg/Rhine-Neckar-County, Würzburg/Lower Frankonia, Hamburg, Bielefeld/Gütersloh, and Munich). For each newly recruited lymphoma case, a gender, region and age-matched (+/-1 year of birth) population control was drawn from the population registers. In a structured personal interview, we elicited a complete occupational history, including every occupational period that lasted at least one year. On the basis of job task-specific supplementary questionnaires, a trained occupational physician assessed the exposure to chlorinated hydrocarbons (trichloroethylene, tetrachloroethylene, dichloromethane, carbon tetrachloride) and aromatic hydrocarbons (benzene, toluene, xylene, styrene). Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression analysis, adjusted for smoking (in pack years) and alcohol consumption. To increase the statistical power, patients with specific lymphoma subentities were additionally compared with the entire control group using unconditional logistic regression analysis. RESULTS: We observed a statistically significant association between high exposure to chlorinated hydrocarbons and malignant lymphoma (Odds ratio = 2.1; 95% confidence interval 1.1–4.3). In the analysis of lymphoma subentities, a pronounced risk elevation was found for follicular lymphoma and marginal zone lymphoma. When specific substances were considered, the association between trichloroethylene and malignant lymphoma was of borderline statistical significance. Aromatic hydrocarbons were not significantly associated with the lymphoma diagnosis. CONCLUSION: In accordance with the literature, this data point to a potential etiologic role of chlorinated hydrocarbons (particularly trichloroethylene) and malignant lymphoma. Chlorinated hydrocarbons might affect specific lymphoma subentities differentially. Our study does not support a strong association between aromatic hydrocarbons (benzene, toluene, xylene, or styrene) and the diagnosis of a malignant lymphoma.

B.3.2.6.3.2. ***Study description and comment***. This population case-control study of NHL and Hodgkin's lymphoma patients in six Germany regions is part of a larger multiple-center and -country case-control study of lymphoma and environmental exposures, the EPILYMPH study. A total of 710 cases and 710 controls that were matched to cases on age, sex, and region, participated in this study. Participation rates were 88% for cases and 44% for controls. Potential for selection bias may exist given the low control response rate. Strength of this study is the use of WHO classification scheme for classifying lymphomas and the high percentage of cases with histologically-confirmed diagnoses. An industrial physician blinded to case and control status assigned exposure to specific solvents (i.e., TCE, perchloroethylene, carbon tetrachloride, etc.) using a JEM developed for the EPILYMPH investigators, a modification of Bolm-Audorff et al. (1988). Exposure prevalence to TCE among cases was 13%. A cumulative exposure score was calculated and was the sum for every job held of intensity of solvent exposure, frequency of exposure, and duration of exposure. High exposure to TCE was defined as >35 ppm-years; 3% of cases had high cumulative exposure to TCE. Intensity of TCE exposure was assessed on a semiquantitative scale with the following categories: low intensity, 2.5 ppm (0.5 to 5); medium intensity, 25 ppm (>5 to 50), high intensity, 100 ppm (>50). The frequency of exposure was the percentage of working time during which the exposure occurred based upon a 40-hour week. A semiquantitative scale was adopted for frequency of exposure with the following categories: low frequency, 3% of working time (range, 1 to 5%), medium frequency, 17.5 % (range, >5 to 30%), high frequency, 65% of working time (>30%). A cumulative Prevalence of TCE exposure among cases was 13% overall with 3% of cases identified with cumulative exposure >35 ppm-years.

Overall, the use of expert assessment for exposure and WHO classification for disease coding likely reduce misclassification bias in this study. This population case-control study, like other population case-control studies of lymphoma and TCE, has a low prevalence of TCE exposure and limits statistical power to detect risk factors.

Seidler A, Mohner M, Berger J, Mester B, Deeg E, Eisner G, Neiters A, Becker N. 2007. Solvent exposure and malignant lymphoma: a population-based case-control study in Germany. J Occup Med Toxicol 2:2. Accessed August 27, 2007, http://www.occup-med.com/content/2/1/2.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This case-control study of NHL and Hodgkin lymphomas was designed to investigate association between specific exposure and distinct lymphoma classifications which are defined by REAL and WHO classifications.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	812 male and female lymphoma patients between the ages of 18 and 80 yrs were identified from a six German study regions from 1999 to 2003. 1,602 controls were identified from population registers and matched (1:1) to cases on sex, region and age. 710 cases and 710 controls were interviewed.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL and Hodgkin's lymphoma incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	WHO classification. Diagnosis confirmed by pathological report for 691 cases.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Blinded assignment of intensity and frequency of exposure to specific chlorinated hydrocarbons (includes TCE) and to aromatic hydrocarbons based upon questionnaire information on complete occupational history for all jobs of $\geq$1 yr duration. Exposure assessment approach based on a modification of Bolm-Audorff et al. (1988).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Face-to-face interview with questionnaire for detailed information about medical history, lifestyle factors, and occupation. Job-task-specific supplementary questionnaire administered to subjects having held jobs of interest; e.g., painters, metal workers and welders, dry cleaners, chemical workers, shoemakers and leather workers, and textile workers.
Blinded interviewers	Unblinded interviews.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information provided in paper.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	710 cases (87.4%) and 710 controls (44.3%). Exposure prevalence: Any TCE exposure, Cases, 13%, Controls, 15%. High cumulative exposure (>35 ppm-yr), Cases, 3%, Controls, 1%.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, region, pack years of smoking, and # grams of alcohol consumed per day.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes, by ppm-yr as continuous variable.
Documentation of results	Yes.

B.3.2.6.4. ***Persson and Fredrikson (1999), Persson et al. (1993, 1989).***

B.3.2.6.4.1. ***Author's abstract.***

> Non-Hodgkin's lymphoma (NHL) has been subject to several epidemiological studies and various occupational and non-occupational exposures have been identified as determinants. The present study is a pooled analysis of two earlier methodologically similar case-referent studies encompassing 199 cases of NHL and 479 referents, all alive. Exposure information, mainly on occupational agents, was obtained by mailed questionnaires to the subjects. Exposure to white spirits, thinner, and aviation gasoline as well as work as a painter was connected with increased odds ratios, whereas no increased risk was noted for benzene. Farming was associated with a decreased odds ratio and exposure to phenoxy herbicides, wood preservatives, and work as a lumberjack showed increased odds ratios. Moreover, exposure to plastic and rubber chemicals and also contact with some kinds of pets appeared with increased odds ratios. Office employment and housework showed decreased odds ratios. This study indicates the importance of investigating exposures not occurring very frequently in the general population. Solvents were studied as a group of compounds but were also separated into various specific compounds. The present findings suggest that the carcinogenic property of solvents is not only related to the aromatic ones or to the occurrence of benzene contamination, but also to other types of compounds.

B.3.2.6.4.2. ***Study description and comment***. The exposure assessment approach of Persson and Fredriksson (1999), a pooled analysis of NHL cases and referents in Persson et al. (1993) and Persson et al. (1989), was based upon self-reported information obtain from a mailed questionnaire to cases and controls. Ten of 17 main questions of the detailed multiple-page questionnaire concerned occupational exposure, with additional questions on specific job and exposure details. These studies of the Swedish population considered exposure durations of 1 or more years and those received 5 to 45 years before NHL diagnosis for cases and before the point in time of selection for controls. The period of TCE exposure assessed in the between 1964 and 1986, a time period similar to that of Axelson et al. (1994). Semiqualitative information about solvent exposure was obtained directly from the questionnaires. Assignment of exposure potential to individual solvents such as TCE and white spirit is not described nor does the paper describe whether assignment was done blinded as to case or control status. A five-category classification for intensity was developed although statistical analyses grouped the TCE categories as intensity scores of >2 compared to 0/1. TCE exposure prevalence among cases was 8% (16 of 199) and 7% among referents (32 of 479).

This small study of 199 NHL cases diagnosed between 1964 and 1986 at a regional Swedish hospital (Orebro) and alive at the time of data acquisition in 1986 was similar in design to other lymphoma (chronic lymphocytic leukemia, multiple myeloma) and occupation studies from these investigators (Flodin et al., 1987, 1988). A series of 479 referents from the same catchment area and from the same time period, identified previously from the multiple myeloma and chronic lymphocytic leukemia studies, served as the source for controls in Persson and Fredrikson (1999) for the NHL analysis and in Persson et al. (1989, 1993) for the Hodgkin's lymphoma analysis. Given the study's entrance date as 1964, with interviews carried out in the 1980s, some cases were deceased with information likely provided by proxy respondents. The paper does not identify the percentage of deceased cases and the magnitude of potential bias associated with proxy respondents can not be determined. Little information is provided in the published paper on controls; however, the paper notes 17% of eligible controls were not able or unwilling to respond to the questionnaire. Case and control series appear to differ given only subjects 40 to 80 years of age were included in the statistical analysis. Cases in Perrson et al. (1993) were histologically confirmed diagnosis of NHL; this was not so for Persson et al. (1989). Misclassification associated with misdiagnosis is not expected to be large given observation in Perrson et al. (1993) of 2% of lymphoma cases were misclassified.

Overall, the study's 20-year period between initial case and control identification and interview suggests some subjects were either survivors or information was obtained from proxy respondents. In both instances, misclassification bias is likely. No information is provided on job titles or the nature of TCE exposure, which was defined in the exposure assessment as "exposed or unexposed." Exposure prevalence to TCE in this study is higher than that found in community population studies of Miligi et al. (2006), Seidler et al. (2007), and Costantini et al. (2008).

Persson B, Fredrikson M. 1999. Some risk factors for non-Hodgkin's lymphoma. Int J Occup Med Environ Health 12:135−142.

Persson B, Fredriksson M, Olsen K, Boeryd B, Axelson O. 1993. Some occupational exposure as risk factors for malignant lymphomas. Cancer 72:1773−1778.

Persson B, Dahlander A-M, Fredriksson M, Brage HN, Ohlson C-G, Axelson O. 1989. Malignant lymphomas and occupational exposures. Br J Ind Med 46:516−520.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	These studies of Hodgkin's Lymphoma and NHL investigated occupational associations. Examination of TCE is not stated as *a priori* hypothesis.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Incident NHL and Hodgkin's lymphoma cases reported to a regional cancer registry between 1975 and 1984, *n* = 148 (Persson et al., 1993), or identified from hospital records (Orebro Medical Center Hospital) for the period 1964 and 1986, *n* = 175 (Persson et al., 1989). Population controls from the same geographical area as cases were identified from previous case-control studies of leukemia and multiple myeloma and matched on age and sex. Analysis of NHL and Hodgkin's lymphoma each used the same set of controls. Persson and Fredrikson (1999)—199 cases of NHL, 479 controls. Persson et al., 1993—93 NHL and 31 Hodgkin's lymphoma (90% participation); 204 controls. Persson et al., 1989—106 NHL and 54 Hodgkin's lymphoma (91%); 275 controls.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Classification system not identified in papers.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported occupational exposures as obtained from a mailed questionnaire.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Mailed questionnaire, only.
Blinded interviewers	N/A
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information provided in paper.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Exposure prevalence to TCE— Persson and Fredrikson (1999)—16 NHL cases (8%) and 32 controls (7%). Persson et al. (1993)—8 NHL cases (8%) and 5 Hodgkin's lymphoma cases (16%); 18 controls (9%). Persson et al. (1989)—8 NHL cases (8%) and 7 Hodgkin's lymphoma cases (13%); 14 controls (5%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and controls are matched on age and sex. Statistical analyses do not control for other possible confounders.
Statistical methods	Only crude odds ratios are presented for TCE exposure, although logistic regression was used to examine other occupational exposure and NHL/Hodgkin's lymphoma.
Exposure-response analysis presented in published paper	No.
Documentation of results	Poor, unable to determine response rate in control population, if controls were similar to cases on demographic variables such as sex and age, and whether controls were identified from same time period as cases.

B.3.2.6.5. *Nordstrom et al. (1998).*

B.3.2.6.5.1. ***Author's abstract.***

> To evaluate occupational exposures as risk factors for hairy cell leukemia (HCL), a population-based case-control study on 121 male HCL patients and 484 controls matched for age and sex was conducted. Elevated odds ratio (OR) was found for exposure to farm animals in general: OR 2.0, 95% confidence interval (CI) 1.2-3.2. The ORs were elevated for exposure to cattle, horse, hog, poultry and sheep. Exposure to herbicides (OR 2.9, CI 1.4-5.9), insecticides (OR 2.0, CI 1.1-3.5), fungicides (OR 3.8, CI 1.4-9.9) and impregnating agents (OR 2.4, CI 1.3-4.6) also showed increased risk. Certain findings suggested that recall bias may have affected the results for farm animals, herbicides and insecticides. Exposure to organic solvents yielded elevated risk (OR 1.5, CI 0.99-2.3), as did exposure to exhaust fumes (OR 2.1, CI 1.3-3.3). In an additional multivariate model, the ORs remained elevated for all these exposures with the exception of insecticides. We found a reduced risk for smokers with OR 0.6 (CI 0.4-1.1) because of an effect among non-farmers.

B.3.2.6.5.2. ***Study description and comment***. This population case-control of hairy cell leukemia, a B-cell lymphoid neoplasm and NHL, examined occupational organic solvent and pesticide exposures among male cases reported to the Swedish Cancer Registry between 1987 and 1992. A total of 121 cases, including 1 case one case, originally thought to have a diagnosis within the study's window, but latter learned as in 1993, and four controls per case matched on age and county of residence from the Swedish Population Registry. Occupational exposure was assessed based upon self-reported information provided in a mailed questionnaire with telephone follow-up by trained interviewer blinded to case or control status. Chemical-specific exposures of at least 1 day duration and occurring one year prior to case diagnosis were assigned to study subjects; however, the procedure for doing this was not described in the paper. Potential for organic solvents exposure included exposure received during leisure activities and work-related activities. Exposure prevalence to TCE among cases is 8 and 7% among controls. The low exposure prevalence and study size limit the statistical power of this study for detecting relative risks smaller than 2.0.

Odds ratios and 95% confidence intervals are presented for chemical-specific exposures, including TCE, from logistic regression models in two separate analyses, univariate analysis and multivariate analysis adjusting for age. The odds ratio for TCE exposure is presented only from univariate analysis. Age may not greatly confound or bias the observed association; an examination of risk estimates from univariate and multivariate analyses of the aggregated exposure category for organic solvents showed similar odds ratios, indicating age was not a significant source of bias in the statistical analyses because age was controlled in the study's design, a control was matching to a case on age.

Nordstrom M, Hardell L, Hagberg H, Rask-Andersen A. 1998. Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukemia evaluated in a case-control study. Br J Cancer 77:2048−2052.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Abstract—To evaluate occupational exposure as risk factors for hairy cell leukemia.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	121 cases of HCL in males reported to the Swedish Cancer Registry between 1987 and 1992. 484 controls (1:4 matching) identified from Swedish Population Registry and matched for age and county of residence.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper, likely ICD-9 (http://www.socialstyrelsen.se/, accessed February 6, 2009).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported information on occupational exposure as obtained from a mailed questionnaire to study participants. Questionnaire sought information on complete working history, other exposures, and leisure time activities with telephone interview in cases of incomplete information. Paper does not describe the procedure for assigning chemical exposures from job title information.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Mailed questionnaire.
Blinded interviewers	Follow-up telephone interview and job/exposure coding were done blinded as to case and control status.

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Proxy responses: 4%, cases; 1% controls.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	111 HCL cases, 400 controls. Response rate: 91% cases and 83% controls. Exposure prevalence among cases is 8 and 7% among controls.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and controls are matched for age, sex, and county of residence. Effect measure for TCE exposure from univariate analysis presented in paper; other possible confounders or covariates not included in statistical analysis.
Statistical methods	Logistic regression.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

HCL = hairy cell leukemia.

B.3.2.6.6. *Fritschi and Siemiatycki (1996a), Siemiatycki (1991).*

B.3.2.6.6.1. ***Author's abstract.***

> The known risk factors for lymphoma and myeloma cannot account for the current incidence rates of these cancers, and there is increasing interest in exploring occupational causes. We present results regarding lymphoma and myeloma from a large case-control study of hundreds of occupational exposures and 19 cancer sites. We examine in more detail those exposures previously considered to be related to these cancers, as well as exposures which were strongly related in our initial analyses. Lymphoma was not associated in our data with exposure to solvents or pesticides, or employment in agriculture or wood-related occupations, although numbers of exposed cases were sometimes small. Hodgkin's lymphoma was associated with exposure to fabric dust, and non-Hodgkin's lymphoma was associated with exposure to copper dust, ammonia and a number of fabric and textile-related occupations and exposures. Employment as a sheet metal worker was associated with development of myeloma.

B.3.2.6.6.2. ***Study description and comment***. This population study of several cancer sites included histologically-confirmed cases of NHL, Hodgkin's lymphoma and myeloma ascertained from 16 Montreal-area hospitals between 1979 and 1985 and part of a larger study of 10 other cancer sites. This study relies on the use of expert assessment of occupational information on a detailed questionnaire and face-to-face interview. Fritschi and Siemiatycki (1996a) present observations of analyses examining industries, occupation, and some chemical-specific exposures, including solvents, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991).

A total of 215 NHL cases (83% response) were identified from 19 Montreal-area hospitals and while this case group is larger than that in Swedish lymphoma case-control studies, there are fewer NHL cases than other multicenter studies published since 2000. The 533 population controls (72% response), identified through the use of random digit dialing, and were used for each site-specific cancer case analyses. All controls were interviewed using face-to-face methods; however, 20% of the NHL cases were either too ill to interview or had died and, for these cases, occupational information was provided by a proxy respondent. The quality of interview conducted with proxy respondents was much lower, increasing the potential for misclassification bias, than that with the subject. The direction of this bias would diminish observed risk towards the null. Interviewers were unblinded, although exposure assignment was carried out blinded as to case and control status. The questionnaire sought information on the subject's complete job history and included questions about the specific job of the employee and work environment. Occupations considered with possible TCE exposure included machinists, aircraft mechanics, and industrial equipment mechanics. An additional specialized questionnaire was developed for certain job title of *a prior* interest that sought more detailed information on tasks and possible exposures. For example, the supplemental questionnaire for machinists included a question on TCE usage.

A team of industrial hygienists and chemicals assigned exposures blinded based on job title and other information obtained by questionnaire. A semiquantitative scale was developed for 294 exposures and included TCE (any, substantial). Any exposure to TCE was 3% among cases but <1% for substantial TCE exposure; "substantial" is defined as $\geq$10 years of exposure for the period up to 5 years before diagnosis. The TCE exposure frequencies in this study are lower than those in more recent NHL case-control studies examining TCE. The expert assessment method is considered a valid and reliable approach for assessing occupational exposure in community-base studies and likely less biased from exposure misclassification than exposure assessment based solely on self-reported information (IOM, 2003; Fritschi et al., 2003; Siemiatycki et al., 1997).

Logistic regression models adjusted for age, ethnicity, income, and respondent status (Fritschi and Siemiatycki, 1996a) or Mantel-Haenszel χ^2 stratified on age, family income, and cigarette smoking (Siemiatycki, 1991). Odds ratios for TCE exposure are presented with 90% confidence intervals in Siemiatycki (1991) and with 95% confidence intervals in Fritschi and Siemiatycki (1996a).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of NHL. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals. Overall, a reasonably good exposure assessment is found in this analysis; however, examination of NHL and TCE exposure is limited by statistical power considerations related to low exposure prevalence, particularly for "substantial" exposure. For the exposure prevalence found in this study to TCE and for NHL, the minimum detectable odds ratio was 3.0 when $\beta = 0.02$ and $\alpha = 0.05$ (one-sided). The low statistical power to detect a doubling of risk and an increased possibility of misclassification bias associated with case occupational histories resulting from proxy respondents suggests this study is less sensitive than other NHL case-controls published since 2000 for examining NHL and TCE.

Fritschi L, Siemiatycki J. 1996a. Lymphoma, myeloma and occupation: Results of a case-control study. Int J Cancer 67: 498−503.

Siemitycki J. 1991. Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study of NHL was designed to investigate association between specific exposure and cancers at 20 sites using expert assessment method for exposure assignment.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	258 histologically-confirmed NHL cases were identified among Montreal area males, aged 35 to 70 yrs, diagnosed in 16 Montreal hospitals between 1979 and 1985. 740 male population controls were identified from the same source population using random digit dialing methods.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	NHL.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICDO-0, 200 and 202 (International Statistical Classification of Diseases for Oncology, WHO, 1997). ICDO-0 is based upon rubrics of ICD, 9th Revision.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 300 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Yes, 82% of case interviews were face-to-face; 100% of control interviews were with subject.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, ~20% of cases had proxy respondents. Interviews were completed with all control subjects.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	215 cases (83% response), 533 population controls (71%). Exposure prevalence: Any TCE exposure, 3% cases; Substantial TCE exposure (Exposure for $\geq$10 yrs and up to 5 yrs before disease onset), <1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, income, index for cigarette smoking (Siemiatycki, 1991). Age, proxy status, income, ethnicity (Fritschi and Siemiatycki, 1996a).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Unconditional logistic regression (Fritschi and Siemiatycki, 1996a).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.6.7. *Hardell et al. (1994, 1981).*

B.3.2.6.7.1. ***Author's abstract.***

> Results on 105 cases with histopathologically confirmed non-Hodgkin's lymphoma (NHL) and 335 controls from a previously published case-control study on malignant lymphoma are presented together with some extended analyses. No occupation was a risk factor for NHL. Exposure to phenoxyacetic acids yielded, in the univariate analysis, an odds ratio of 5.5 with a 95% confidence interval of 2.7-11. Most cases and controls were exposed to a commercial mixture of 2, 4-dichlorophenoxyacetic acid and 2, 4, 5-trichlorophenoxyacetic acid. Exposure to chlorophenols gave an odds ratio of 4.8 (2.7-8.8) with pentachlorophenol being the most common type. Exposure to organic solvents yielded an odds ratio of 2.4 (1.4-3.9). These results were not significantly changed in the multivariate analysis. Dichlorodiphenyltrichloroethane, asbestos, smoking, and oral snuff were not associated with an increased risk for NHL. The results regarding increased risk for NHL following exposure to phenoxyacetic acids, chlorophenols, or organic solvents were not affected by histopathological type, disease stage, or anatomical site of disease presentation. Median survival was somewhat longer in cases exposed to organic solvents than the rest. This was explained by more prevalent exposure to organic solvents in the group of cases with good prognosis NHL histopathology.
>
> A number of men with malignant lymphoma of the histiocytic type and previous exposure to phenoxy acids or chlorophenols were observed and reported in 1979. A matched case-control study has therefore been performed with cases of malignant lymphoma (Hodgkin's disease and non-Hodgkin lymphoma). This study included 169 cases and 338 controls. The results indicate that exposure to phenoxy acids, chlorophenols, and organic solvents may be a causative factor in malignant lymphoma. Combined exposure of these chemicals seemed to increase the risk. Exposure to various other agents was not obviously different in cases and in controls.

B.3.2.6.7.2. ***Study description and comment***. Exposure in these case-control studies of histologically-confirmed lymphoma (NHL and Hodgkin's lymphoma) (Hardell et al., 1981) or only the NHL cases only (Hardell et al., 1994) over a 4-year period, 1974−1978, in Umea, Sweden was assessed based upon information provided in a self-administered questionnaire. The questionnaire obtained information on a complete working history over the life of the subjects along with information on various other exposures and leisure time activities. Organic solvent exposures were examined secondary to this study's primary hypothesis examining phenoxy acid or chlorophenol exposures and lymphoma. The extent of recall bias related to self-reported information can not be determined nor is information provided in the published papers misclassification bias resulting from next-of-kin interviews. Occupations were classification according to the Nordic Working Classification system. Chemical specific exposures assignment was not described but appears to have been carried out blinded as to case or control status. A semiquantitative classification scheme based on intensity and duration of exposure was used to categorize solvent exposure into two groupings: low grade—less than 1 week continuously or less than 1 month in total—and high grade for all other exposure scenarios. TCE exposure prevalence is similar in both studies; 4% for cases and 1% for controls. The low exposure prevalence and small numbers of cases with TCE exposure ($n = 4$) limits the statistical power of these analyses and results in wide confidence intervals around the estimated odds ratio for TCE exposure (95% Confidence Interval, 1.3−42).

The Rappaport Classification was used to identify non-Hodgkin's and Hodgkin's Lymphoma cases. The Rappaport Classification was in widespread use until the 1970s and was based on a cell's pathologic characteristics. Equivalence of non-Hodgkin's lymphoma groupings according to Rappaport Classification system to ICDA-8 groupings, also in use during this time period, is 200 "Lymphosarcoma and reticulum-cell sarcoma" and 202 "Other neoplasms of lymphoid tissue."

Hardell L, Eriksson M, Degerman A. 1994. Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. Cancer Res 54:2386−2389.

Hardell L, Eriksson M, Lenner P, Lundgren E. 1981. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br J Cancer 43:169−176.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	NHL cases from a case-control study of lymphoma (NHL and Hodgkin's lymphoma) are analyzed separately to evaluate herbicide and organic solvents exposure.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	105 cases of histologically-confirmed NHL among males aged 25−85 yrs admitted to local hospital's oncology department between 1974 and 1978. A total of 335 male controls identified from the Swedish Population Registry, for living cases, and from the Swedish Registry for Causes of Death, for dead cases. Controls matched to cases by age, residence municipality, and year of death, for dead cases.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Rappaport Classification; equivalent to ICDA-8 Codes, 200, and 202.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Self-reported information on occupational exposure as obtained by questionnaire, with a telephone interview for incomplete or unclear information. Questionnaire sought information on complete working history, other exposures and leisure time activities. Paper does not describe the procedure for assigning chemical exposures from job title information.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	No information in paper.
Blinded interviewers	Follow-up telephone interview was done blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information in paper.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	105 NHL cases, 332 controls. Response rates could not be calculated given insufficient information in paper. Prevalence of TCE exposure, 4% cases, 1% controls.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and controls matched on sex, age, place of residence and vital status. For deceased controls are matched to deceased cases on year of death.
Statistical methods	Mantel-Haenszel stratified by age and vital status.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.7. *Childhood Leukemia*

B.3.2.7.1. *Shu et al. (2004, 1999).*

B.3.2.7.1.1. ***Author's abstract.***

> Ras proto-oncogene mutations have been implicated in the pathogenesis of many malignancies, including leukemia. While both human and animal studies have linked several chemical carcinogens to specific ras mutations, little data exist regarding the association of ras mutations with parental exposures and risk of childhood leukemia. Using data from a large case control study of childhood acute lymphoblastic leukemia (ALL; age <15 years) conducted by the Children's Cancer Group, we used a case-case comparison approach to examine whether reported parental exposure to hydrocarbons at work or use of specific medications are related to ras gene mutations in the leukemia cells of children with ALL. DNA was extracted from archived bone marrow slides or cryopreserved marrow samples for 837 ALL cases. We examined mutations in K-ras and N-ras genes at codons 12, 13, and 61 by PCR and allele-specific oligonucleotide hybridization and confirmed them by DNA sequencing. We interviewed mothers and, if available, fathers by telephone to collect exposure information. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from logistic regression to examine the association of parental exposures with ras mutations. A total of 127 (15.2%) cases had ras mutations (K-ras 4.7% and N-ras 10.68%). Both maternal (OR 3.2, 95% CI 1.7-6.1) and paternal (OR 2.0, 95% CI 1.1-3.7) reported use of mind-altering drugs were associated with N-ras mutations. Paternal use of amphetamines or diet pills was associated with N-ras mutations (OR 4.1, 95% CI 1.1-15.0); no association was observed with maternal use. Maternal exposure to solvents (OR 3.1, 95% CI 1.0-9.7) and plastic materials (OR 6.9, 95% CI 1.2-39.7) during pregnancy and plastic materials after pregnancy (OR 8.3, 95% CI 1.4-48.8) were related to K-ras mutation. Maternal ever exposure to oil and coal products before case diagnosis (OR 2.3, 95% CI 1.1-4.8) and during the postnatal period (OR 2.2, 95% CI 1.0-5.5) and paternal exposure to plastic materials before index pregnancy (OR 2.4, 95% CI 1.1-5.1) and other hydrocarbons during the postnatal period (OR 1.8, 95% CI 1.0-1.3) were associated with N-ras mutations. This study suggests that parental exposure to specific chemicals may be associated with distinct ras mutations in children who develop ALL.
>
> Parental exposure to hydrocarbons at work has been suggested to increase the risk of childhood leukemia. Evidence, however, is not entirely consistent. Very few studies have evaluated the potential parental occupational hazards by exposure time windows. The Children's Cancer Group recently completed a large-scale case-control study involving 1842 acute lymphocytic leukemia (ALL) cases and 1986 matched controls. The study examined the association of self-reported occupational exposure to various hydrocarbons among parents with risk of childhood ALL by exposure time window, immunophenotype of ALL, and age at diagnosis. We found that maternal exposure to solvents [odds ratio (OR), 1.8; 95% confidence interval (CI), 1.3-2.5] and paints or thinners (OR, 1.6; 95% CI, 1.2-2.2) during the preconception period (OR, 1.6; 95% CI, 1.1-2.3) and during

pregnancy (OR, 1.7; 95% CI, 1.2-2.3) and to plastic materials during the postnatal period (OR, 2.2; 95% CI, 1.0-4.7) were related to an increased risk of childhood ALL. A positive association between ALL and paternal exposure to plastic materials during the preconception period was also found (OR, 1.4; 95% CI, 1.0-1.9). The ALL risk associated with parental exposures to hydrocarbons did not vary greatly with immunophenotype of ALL. These results suggest that the effect of parental occupational exposure to hydrocarbons on offspring may depend on the type of hydrocarbon and the timing of the exposure.

B.3.2.7.1.2. ***Study description and comment***. Parent hydrocarbon occupational exposure in this case-control study of acute lymphatic leukemia in children less than 15 years of age was assessed from telephone questionnaire to mothers and, whenever available, fathers of cases and controls who were part of the large-scale incidence study by the Children's Cancer/Oncology Group. A recent paper examines hydrocarbon exposures and relationship with the ras proto-oncogene (Shu et al., 2004). Nearly 50% of childhood leukemia cases in the United States were treated by a Children's Cancer Group hospital or institution and between January 1, 1989 and June 15, 1993, the study period, a total of 2,081 incident childhood leukemia cases were identified with 1,914 interviews with mothers. Controls were randomly selected using a random digit dialing procedure and matched to cases on age, race, and geographic location. Using structured questionnaires, parents or a surrogate when unavailable were asked about job title, industry, duties, starting and stopping date for all jobs held by the father for more than 6 months beginning at age 18 years and by the mother for all jobs held at least 6 months in the period from 2 year prior to the index pregnancy to date of diagnosis of leukemia case or the reference date of the controls. The questionnaire sought information on specific exposures to solvents (carbon tetrachloride, TCE, benzene, toluene, and xylene), plastic materials, paints, pigments or thinners, and oil or coal products. Exposure quantitative was not possible. Statistical analyses use self-reported exposure to specific hydrocarbons as defined as a dichotomous variable (yes/no). The potential for misclassification bias is greater with exposure assessment based upon self-reports compared to that by expert assessment (Teschke et al., 2002). Exposure information was linked to start and stop data of the relevant job to determine the timing of exposure related to specific windows of possible susceptibility for acute lymphoblastic leukemia (ALL). The author's do not describe jobs associated with possible TCE exposure.

The father's questionnaire was completed for 1,801 of the 2,081 eligible cases and 1,813 of the 2,597 eligible controls. Of the 1,618 matched sets, direct interview with fathers were obtained for 83% of cases and 68% of controls. Maternal interview were completed for 1,914 of the 2,081 eligible cases (92%). The low prevalence of any exposure to TCE, 1% for mothers (15 cases of 1,842 matched pairs with maternal exposure information) and 8% for fathers

(136 cases out 1,618 matched pairs), limits the statistical power of this study to detect low to moderate risk.

Shu Xo, Perentesis JP, Wen W, Buckley JD, Boyle E, Ross, JA, Robison LL. 2004. Parental exposure to medications and hydrocarbons and ras mutations in children with acute lymphoblastic leukemia: A report from the Children's Oncology Group. Cancer Epidemiol Biomarkers Prev 13:1230−1235.

Shu XO, Stewart P, Wen W-Q, Han D, Potter JD, Buckley JD, Heineman E, Robison LL. 1999. Parental occupational exposure to hydrocarbons and risk of acute lymphocytic leukemia in offspring. Cancer Epidemiol Markers Prev 8:783−291.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Shu et al. (1999, 2004) examine possible association with a number of maternal and paternal exposures among cases and controls identified from the Children's Cancer/Oncology Group. The Children's Cancer/Oncology Group is an association of more than 120 centers in the United States, Canada, and Australia who collaboratively carry out research on risk factors and treatment of childhood cancers.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	848 children with acute lymphatic leukemia of ages 0−9 yrs of age at diagnosis from 1980−1993 and ≤14 yrs old at diagnosis between 1994 and 2000 were identified from cancer care centers in Québec, Canada. Controls are concurrently identified from population, from 1980−1993, from family allowance files and from 1994−2000, from universal health insurance files; and, matched (1:1 matching ratio) to cases on sex and age at the time of diagnosis (calendar date). Participation rates- 93.1% cases (790 of 849 eligible cases); 86.2% controls (790 of 916 eligible controls).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Childhood leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD, 9th revision, Code 204.0.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Telephone interviews of mothers of cases and controls using structured questionnaire were administered to obtain information on general risk factors and potential confounders. Questionnaire also sought information on a complete job history, for the mother from 18 years of age to the end of pregnancy and included for each job, job title, dates of employment, type of industry, and location of employer. Statistical analyses based on self-reported occupational exposure to hydrocarbons as defined by broad groups and individual hydrocarbons.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interview, >99% response.
Blinded interviewers	Telephone interviews were not blinded, but exposure assignment and coding was carried out blinded to case and control status by chemists and industrial hygienists.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	100% of cases and controls had maternal history provided by direct interview with mothers. 13% of cases and 30% of controls had paternal information provided by proxy respondent (e.g., through maternal interview).
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	15 cases (2% exposure prevalence) and 9 controls (1% exposure prevalence) with maternal TCE exposure. 136 cases (8% exposure prevalence) and 104 controls (13% exposure prevalence) with paternal TCE exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Child's age at time of diagnosis , sex, and calendar year of diagnosis, maternal age and level of schooling.

Statistical methods	Conditional logistic regression— By two time periods; 2 yrs before pregnancy up to birth, during specific pregnancy period. By level of exposure; Level 1 (some exposure) compared to no exposure, and Level 2 (greater exposure potential) compared to no exposure.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.7.2. *Costas et al. (2002), MADPH (1997).*

B.3.2.7.2.1. ***Author's abstract.***

A 1981 Massachusetts Department of Public Health study confirmed a childhood leukemia cluster in Woburn, Massachusetts. Our follow-up investigation attempts to identify factors potentially responsible for the cluster. Woburn has a 130-year industrial history that resulted in significant local deposition of tannery and chemical manufacturing waste. In 1979, two of the city's eight municipal drinking water wells were closed when tests identified contamination with solvents including trichloroethylene. By 1986, 21 childhood leukemia cases had been observed (5.52 expected during the seventeen year period) and the case-control investigation discussed herein was begun. Nineteen cases and 37 matched controls comprised the study population. A water distribution model provided contaminated public water exposure estimates for subject residences. Results identified a non-significant association between potential for exposure to contaminated water during maternal pregnancy and leukemia diagnosis, (odds RATIO=8.33, 95% CI 0.73–94.67). However, a significant dose-response relationship ($P<0.05$) was identified for this exposure period. In contrast, the child's potential for exposure from birth to diagnosis showed no association with leukemia risk. Wide confidence intervals suggest cautious interpretation of association magnitudes. Since 1986, expected incidence has been observed in Woburn including 8 consecutive years with no new childhood leukemia diagnoses.

B.3.2.7.2.2. ***Study description and comment***. Exposure in this case-control study of childhood leukemia over a 20-year period in Woburn, MA was assessed based upon the potential for a residence at the time of diagnosis to receive water from wells G and H, wells with a hydraulic mixing model of Murphy (1991) which described the town's water distribution system. Monitoring of wells G and H in 1979 showed the presence of several VOCs; TCE and perchloroethylene (PERC) were found to exceed drinking water guidelines, at 267 ppb and 21 ppb, respectively. Low levels of other contaminates were detected including chloroform, 1,2-dichloroethylene methyl chloroform, trichlorotrifluoroethane, and inorganic arsenic. The Murphy model described the water flow through Woburn during the lifetime of wells G and H. The model uses data describing the physical layout of Woburn's municipal water system and information regarding the pumping cycles of wells G and H and other active uncontaminated wells that supplied the municipal water system. Model accuracy showed distribution of water from wells G and H to a block area with predicted mixture concentrations with an average error within 10% of the know concentration. Nearly 70% of the model predictions were within 20% of the know validation concentrations. An exposure value for cases and controls by exposure period was the sum of the model-predicted water concentration for each residence in Woburn as assigned to a hydrologically-distinct area along the water distribution network. Both cumulative and average exposure estimates were derived using the model.

Costas K, Knorr RS, Condon SK. 2002. A case-control study of childhood leukemia in Woburn, Massachusetts: the relationship between leukemia incidence and exposure to public drinking water. Sci Total Environ 300:23−25.

Massachusetts Department of Public Health (MADPH). 1997. Woburn Childhood Leukemia Follow-up Study. Volumes I and II. Final Report.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, "this follow-up investigation attempts to identify factors potentially responsible for the leukemia cluster in Woburn, MA" and the primary exposure of concern for investigation is "the potential consumption of contaminated water from Wells G and H by Woburn residents."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	21 cases of leukemia diagnosed in children <19 yrs between 1969 and 1989 who were residents of Woburn MA. Cases diagnosed from 1982 and latter were provided by the Massachusetts Cancer Registry. Cases diagnosed prior to 1982 were identified from local pediatric health professionals and by contacting all greater-Boston childhood oncology centers that treated children with leukemia. Two controls for each case were randomly selected from Woburn Public School records on a geographically basis and matched to cases on race, sex and date of birth ($\pm$3 mos).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Childhood leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O (Acute Lymphatic Leukemia, Acute Myelogenous Leukemia, and Chronic Myelogenous Leukemia).

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	In-person interviewers with mothers and fathers of cases and controls using questionnaire to gather information regarding demographics, residential information for the mother and child, occupational history, maternal medical and reproductive history, child's medical history, and life-style questions. The father's questionnaire contained questions concerning military and occupational history and also included duplicate questions on maternal occupational history, child's medical history and life-style habits. A hydraulic mixing computer model describing Woburn's water distribution system was utilized to assign an exposure index expressed as cumulative number of months a household received contaminated drinking water from Wells G and H. Exposure Index = fraction of time during month when water from Wells G and H reached the user area + fraction of water from Wells G and H supplied to user area. No quantitative measures of TCE and other volatile organic solvents concentrations were included in hydraulic mixing model.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Personal interviews with cases and controls; 19 of 21 cases (91%) and 38 of possible 54 controls (70%) were interviewed.
Blinded interviewers	Interviewers were not blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	One parent interviewed for 21% of cases and 11% of controls.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Participation rates- 93.1% cases (790 of 849 eligible cases); 86.2% controls (790 of 916 eligible controls).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Composite covariates used to control for socioeconomic status, maternal smoking during pregnancy, maternal age at birth of child, and maternal alcohol consumption during pregnancy.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes and includes information in MADPH Final Report (1997).

B.3.2.7.3. *McKinney et al. (1991).*

B.3.2.7.3.1. ***Author's abstract.***

> OBJECTIVE--To determine whether parental occupations and chemical and other specific exposures are risk factors for childhood leukemia. DESIGN--Case-control study. Information on parents was obtained by home interview. SETTING--Three areas in north England: Copeland and South Lakeland (west Cumbria); Kingston upon Hull, Beverley, East Yorkshire, and Holderness (north Humberside), and Gateshead. SUBJECTS--109 children aged 0-14 born and diagnosed as having leukemia or non-Hodgkin's lymphoma in study areas during 1974-88. Two controls matched for sex and date and district of birth were obtained for each child. MAIN OUTCOME MEASURES--Occupations of parents and specific exposure of parents before the children's conception, during gestation, and after birth. Other adults living with the children were included in the postnatal analysis. RESULTS--Few risk factors were identified for mothers, although preconceptional association with the food industry was significantly increased in case mothers (odds ratio 2.56; 95% confidence interval 1.32 to 5.00). Significant associations were found between childhood leukemia and reported preconceptional exposure of fathers to wood dust (2.73, 1.44 to 5.16), radiation (3.23, 1.36 to 7.72), and benzene (5.81, 1.67 to 26.44); ionizing radiation alone gave an odds ratio of 2.35 (0.92 to 6.22). Raised odds ratios were found for paternal exposure during gestation, but no independent postnatal effect was evident. CONCLUSION--These results should be interpreted cautiously because of the small numbers, overlap with another study, and multiple exposure of some parents. It is important to distinguish periods of parental exposures; identified risk factors were almost exclusively restricted to the time before the child's birth.

B.3.2.7.3.2. ***Study description and comment***. A population case-control study of ALL and NHL in children of <14 years of age and residing in three areas in the United Kingdom was carried out to identify possible risk factors for the region's observed increased background childhood leukemia rates. The Sellafield nuclear reprocessing plant was located in one of the areas and one hypothesis was an examination of parental radiation exposure and childhood lymphoma. Un-blinded face-to-face interviews with cases, identified from regional tumor registries, and controls, identified using regional birth registers, used a structured questionnaire to ascertain a complete history of employment and exposure to specific substances and radiation from both child's biological parents, preferred, although, in the absence of one parent, surrogate information by the other parent was obtained from the date of first employment to end of the study period or, if earlier, the date the parent ceased seeing the child. The questionnaire additionally sought information on maternal and paternal exposure to 22 known chemical carcinogens. McKinney et al. (1991) noted that exposures were highly correlated. Information on job title and industry as reported in the questionnaire was coded independently by experts to occupational groupings and titles using a national classification scheme from the Office of Population Census and Surveys and is a strength of this study. The category of metal refining industry and occupations was one of nine occupational groups identified a priori for hypothesis testing. Statistical analyses are based on exposure as defined by industry, occupational title, or chemical-specific exposure.

Interviewers with one or both parents were carried out for 109 of 151 eligible cases (72%) and with 206 of 269 eligible controls (77%), and the low exposure prevalence; no information was presented on the number of surrogate interviews, or, where only one parent responded for both parents. The low prevalence of TCE exposure, 5 discordant pairs (one subject with exposure and the matched subject without exposure) identified with maternal TCE exposure and 16 discordant pairs with paternal preconceptional TCE exposure, greatly limited the statistical power of this study.

McKinney PA, Alexander FE, Cartwright RA, Parker L. 1991. Parental occupations of children with leukemia in west Cumbria, north Humberside, and Gateshead. BMJ 302:681−687.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study examines a number of risk factors (specific chemicals and occupational groups) as possibly associated with the high background rate of acute lymphatic leukemia and non-Hodgkin's lymphoma in children $\leq$14 yrs in the three regions. 22 individual chemicals and 7 occupational groups for *a priori* hypothesis testing.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	151 case children identified from two tumor registries (Yorkshire and Northern Region). No information provided in paper on reporting accuracy of these registries. 269 population controls identified from District health authority birth registers and matched to cases on age, sex, and region of residency at time of case diagnosis. Participation rates- 72% of cases (n = 109) and 77% of controls (n = 206).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Childhood leukemia incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	No information provided in published paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Face-to-face interviews of mothers of cases and controls using structured questionnaire were administered to obtain information on general risk factors and potential confounders. Questionnaire also sought information on a maternal and paternal complete job history, from first employment to end of study and included for job title, dates of employment, and industry. Questionnaire administered to both parents, and, if one parent was unavailable, information was provided by proxy. Questionnaire also sought information on 22 specific chemicals. Expert assignment of occupation based upon National classification system. Statistical analyses industry of employment, job or occupation, and specific exposures.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	No, face-to-face interview with 72% of case parents and 77% of control parents.
Blinded interviewers	Face-to-face interviews were not blinded. Expert assignment of occupation was carried out blinded.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information provided in paper on percentage of proxy interviews.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Exposure prevalence to TCE—maternal exposure, 2 cases (2%) and 3 controls (2%); paternal exposure, 9 cases (9%) and 7 controls (4%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Cases and control matched on age, sex, and region of residency at time of case diagnosis.
Statistical methods	Discordant pair analysis.
Exposure-response analysis presented in published paper	No.
Documentation of results	Limited reporting of odds ratios for job title and occupations.

B.3.2.7.4. *Lowengart et al. (1987).*

B.3.2.7.4.1. ***<u>Author's abstract.</u>***

> A case-control study of children of ages 10 years and under in Los Angeles County was conducted to investigate the causes of leukemia. The mothers and fathers of acute leukemia cases and their individually matched controls were interviewed regarding specific occupational and home exposures as well as other potential risk factors associated with leukemia. Analysis of the information from the 123 matched pairs showed an increased risk of leukemia for children whose fathers had occupational exposure after the birth of the child to chlorinated solvents [odds ratio (OR) = 3.5, P = .01], spray paint (OR = 2.0, P = .02), dyes or pigments (OR = 4.5, P = .03), methyl ethyl ketone (CAS: 78-93-3; OR = 3.0, P = .05), and cutting oil (OR = 1.7, P = .05) or whose fathers were exposed during the mother's pregnancy with the child to spray paint (OR = 2.2, P = .03). For all of these, the risk associated with frequent use was greater than for infrequent use. There was an increased risk of leukemia for the child if the father worked in industries manufacturing transportation equipment (mostly aircraft) (OR = 2.5, P = .03) or machinery (OR = 3.0, P = .02). An increased risk was found for children whose parents used pesticides in the home (OR = 3.8, P = .004) or garden (OR = 6.5, P = .007) or who burned incense in the home (OR = 2.7, P = .007). The risk was greater for frequent use. Risk of leukemia was related to mothers' employment in personal service industries (OR = 2.7, P = .04) but not to specified occupational exposures. Risk related to fathers' exposure to chlorinated solvents, employment in the transportation equipment-manufacturing industry, and parents' exposure to household or garden pesticides and incense remains statistically significant after adjusting for the other significant findings.

B.3.2.7.4.2. ***<u>Study description and comment</u>***. Self-assessed parental exposure to chemical classes and to individual chlorinated solvents was assigned in this case-control study of leukemia in children 10 years or younger using information obtained through telephone interviews with mothers and fathers of cases and controls. Interviews were carried out for 79% of case mothers (159 or 202 cases) and 81% (124 of 154) case fathers. The number of potential controls was not identified in the paper, although it was reported that interviews were carried out for 136 referent mothers and 87 referent fathers. Mothers served as proxy respondents for paternal exposures in roughly 20% of cases and 30% of controls. The complete occupational history was sought for the period 1 year before the case diagnosis date, if the case was older than 2 years, 6 months before the diagnosis date, if the case was between the ages of 1 and 2 years, and the same as the date of diagnosis of the case was <1 year old. Questions on specific occupational exposures such as solvents or degreasers, metals, and other categories were included on the questionnaire, with self-reported information used to assign exposure potential. Exposure is defined only as a dichotomous variable (yes/no). In this study using a matched-pair design in the statistical analyses, there were six case-control pairs of paternal cases but not controls and 3 case-control pairs with paternal controls but not cases with TCE exposure before pregnancy or during pregnancy. Few mothers reported exposure to chlorinated solvents. A strength of the study is the ability to examine exposure at a number of developmental periods, preconception, during pregnancy, and postnatal. Misclassification bias is likely strong in this study, introduced through the large number of proxy respondents and exposure assessment based upon self-reported information. Misclassification resulting from proxy information will dampen observed risks, where as, misclassification of self-reported exposures may bias observed risks in either direction. For this reason and because of the low prevalence of exposure nature of exposure assessment approach, this study provides little information on childhood leukemia risks and TCE exposure.

Lowengart RA, Peters JM, Cicioni C, Buckley J, Bernstein L, Preston-Martin S, Rappaport E. 1987. Childhood leukemia and parents' occupational and home exposures. JNCI 79:39−46.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This case-control study of children ≤10 yrs of age was conducted to identify possible risk factors of childhood leukemia. TCE exposure was one of many occupational exposures assessed in this study.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	202 cases of acute lymphatic leukemia in children ≤10 yrs of age at time of diagnosis from 1980 through 1984 were identified from the Los Angeles County Cancer Surveillance Program, a population-based cancer registry. Controls were identified from among friends of cases with additional controls selected using random digit dialing from the same population as cases and were matched to cases on age, sex, race, and Hispanic origin. 123 cases (61% response rate) and 123 controls (not able to calculate response rate since number of possible controls not identified in paper).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Telephone questionnaire sought information on maternal and paternal preconception, pregnancy, and postnatal (up to 1 yr before case diagnosis) exposures, including a full occupational history (job title, employers, and dates of employments) and on the child's exposure from birth to 1 yr before case diagnosis. Parents also provide self-reported information on specific exposures or occupational activities. Occupations grouped according to hydrocarbon exposure potential using definition of Zack et al. (1980).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interview with 159 of 202 (79%) case mothers and 124 of 202 case fathers (61%). Of controls, interviews were obtained from 136 mothers (65 friends of cases, 71 population controls) and 87 fathers.
Blinded interviewers	Unblinded interviews.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 19% of paternal exposure information on cases was provided by the mother. 43 of 130 control mothers provided information on paternal exposures (33%).
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Paternal TCE exposure— 1 yr before pregnancy, 1/0 discordant pairs During pregnancy, 6/3 discordant pairs After delivery 8/3 discordant pairs. No information is provided in paper on maternal TCE exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, race, and Hispanic origin.
Statistical methods	Discordant pair analysis.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.8. *Melanoma Case-Control Studies*

B.3.2.8.1. *Fritschi and Siemiatycki (1996b), Siemiatycki (1991).*

B.3.2.8.1.1. ***Author's abstract.***

> OBJECTIVES: Associations between occupational exposures and the occurrence of cutaneous melanoma were examined as part of a large population based case-control study of 19 cancer sites. METHODS: Cases were men aged 35 to 70 years old, resident in Montreal, Canada, with a new histologically confirmed cutaneous melanoma (n = 103). There were two control groups, a randomly selected population control group (n = 533), and a cancer control group (n = 533) randomly selected from among subjects with other types of cancer in the large study. Odds ratios for the occurrence of melanoma were calculated for each exposure circumstance for which there were more than four exposed cases (85 substances, 13 occupations, and 20 industries) adjusting for age, ethnicity, and number of years of schooling. RESULTS: Significantly increased risk of melanoma was found for exposure to four substances (fabric dust, plastic dust, trichloroethylene, and a group containing paints used on surfaces other than metal and varnishes used on surfaces other than wood), three occupations (warehouse clerks, salesmen, and miners and quarrymen), and two industries (clothing and non-metallic mineral products). CONCLUSIONS: Most of the occupational circumstances examined were not associated with melanoma, nor is there any strong evidence from previous research that any of those are risk factors. For the few occupational circumstances which were associated in our data with melanoma, the statistical evidence was weak, and there is little or no supporting evidence in the scientific literature. On the whole, there is no persuasive evidence of occupational risk factors for melanoma, but the studies have been too small or have involved too much misclassification of exposure for this conclusion to be definitive.

B.3.2.8.1.2. ***Study description and comment*.** Fritschi and Siemiatycki (1996b) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and melanoma conducted in Montreal, Quebec (Canada) and part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 124 newly diagnosed cases of melanoma (ICD-O, 172), confirmed on the basis of histology reports, between 1979 and 1985; 103 of these participated in the study interview (83.1% participation). One control group (n = 533) consisted of patients with other forms of cancer recruited through the same study procedures and time period as the melanoma cancer cases. A population-based control group (n = 533, 72% response), frequency matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Fritschi and Siemiatycki (1996b) present observations of logistic regression analyses examining industries, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). Any exposure to TCE was 6% among cases ($n = 8$) and 4% for substantial TCE exposure ($n = 4$); "substantial" is defined as $\geq$10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, ethnic origin, socioeconomic status, Quetlet as an index of body mass, and respondent status (Fritschi and Siemiatycki, 1996b) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, Quetlet, ethnic origin, and respondent status (Siemiatycki, 1991). Odds ratios for TCE exposure are presented with 90% confidence intervals in Siemiatycki (1991) and 95% confidence intervals in Fritschi and Siemiatycki (1996b).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of melanoma. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Fritschi L, Siemiatycki J. 1996b. Melanoma and occupation: Results of a case-control study. 1996. Occup Environ Med 53:168−173.

Siemitycki J. 1991. Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	124 melanoma cases were identified among male Montreal residents between 1979 and 1985 of which 103 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of other cancer cases identified in the larger study (*n* = 533). Participation rate: cases, 83.1%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O, 172 (Malignant neoplasm of skin).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	99 cases (76.7% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 8% cases (n = 8); Substantial TCE exposure (Exposure for $\geq$10 yrs and up to 5 yrs before disease onset), 4% cases (n = 4).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, education, and ethnic origin (Fritschi and Siemiatycki, 1996b). Age, family income, cigarette smoking, and ethnic origin (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Fritschi and Siemiatycki, 1996b).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.9. *Pancreatic Cancer Case-Control Studies*

B.3.2.9.1. *Kernan et al. (1999).*

B.3.2.9.1.1. ***Author's abstract.***

> Background The relation between occupational exposure and pancreatic cancer is not well established. A population-based case-control study based on death certificates from 24 U.S. states was conducted to determine if occupations/ industries or work-related exposures to solvents were associated with pancreatic cancer death.
> Methods The cases were 63,097 persons who died from pancreatic cancer occurring in the period 1984±1993. The controls were 252,386 persons who died from causes other than cancer in the same time period.
> Results Industries associated with significantly increased risk of pancreatic cancer included printing and paper manufacturing; chemical, petroleum, and related processing; transport, communication, and public service; wholesale and retail trades; and medical and other health-related services. Occupations associated with significantly increased risk included managerial, administrative, and other professional occupations; technical occupations; and sales, clerical, and other administrative support occupations.
> Potential exposures to formaldehyde and other solvents were assessed by using a job exposure matrix developed for this study. Occupational exposure to formaldehyde was associated with a moderately increased risk of pancreatic cancer, with ORs of 1.2, 1.2, 1.4 for subjects with low, medium, and high probabilities of exposure and 1.2, 1.2, and 1.1 for subjects with low, medium, and high intensity of exposure, respectively.
> Conclusions The findings of this study did not suggest that industrial or occupational exposure is a major contributor to the etiology of pancreatic cancer. Further study may be needed to confirm the positive association between formaldehyde exposure and pancreatic cancer.

B.3.2.9.1.2. ***Study description and comment***. Kernan et al. (1999) reported data from a case-control study of occupational exposures and pancreatic cancer, coding usual occupation as noted on death certificates to assign potential TCE exposure to cases and controls. Deaths from pancreatic cancer from 1984-1993 were identified from 24 U. S. state and frequency-matched to nonpancreatitis or other pancreatic disease deaths by state, race, sex, and age (5-year groups); 63,097 pancreatic cancer deaths (case series) and 252,386 controls were selected for analysis.

Exposure assessment in this study group occupational (n = 509) and industry (n = 231) codes into 16 broad occupational and 20 industrial categories. Additionally, a job exposure matrix (JEM) of Gomez et al. (1994) was applied to develop exposure surrogates for 11 chlorinated hydrocarbons, including TCE, and two larger groupings, all chlorinated hydrocarbons and organic solvents. A qualitative surrogate (ever exposed/never exposed) for

TCE exposure is developed and no information is provided on death certifications on employment duration to examine exposure-response patterns. Kernan et al. (1999) report mortality odds ratios from logistic regression for TCE exposure intensity and probability of exposure.

Overall, this is a large study that examined specific exposures using a generic JEM. Errors resulting from exposure misclassification are likely, not only introduced by the generic JEM, but through the use of usual occupation as coded on death certificates, which may not fully represent an entire occupational history.

Kernan GJ, Ji B-T, Dosemeci M, Silverman DT, Balbus J, Zahm SH. 1999. Occupational risk factors for pancreatic cancer: A case-control study based on death certificates from 24 U. S. states. Am J Ind Med 36:260-270.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between pancreatic cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	63,097 pancreatic cancer cases were identified using death certificates from 24 U. S. states between 1984 and 1993. 63,097 noncancer, nonpancreatitis or other pancreatic disease deaths (controls) identified from the same source population and frequency-matched to cases by state, race, sex, and age (1:4 matching).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-9, 157 (Malignant neoplasm of pancreas).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Usual occupation coded on death certificate coded to 1980 U. S. census classification system for occupation and industry. 509 occupation codes and 231 industry codes grouped into 16 broad occupational and 20 industrial categories based on similarity of occupational exposures. Job exposure matrix of Gomez et al. (1994) used to assign exposure surrogates for 11 chlorinated hydrocarbons, including TCE, and 2 broad categories, chlorinated hydrocarbons and organic solvents.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	This study did not use interviews, information reported on death certificate used to infer potential exposure.
Blinded interviewers	No interviews were conducted in this study.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	Exposure prevalence: Any TCE exposure (Low intensity exposure or higher), 14% cases (n = 9,068); High TCE exposure, 2% cases (n = 1,271).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, metropolitan status, region of residence, and martial status.
Statistical methods	Logistic regression.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.10. *Prostatic Cancer Case-Control Studies*

B.3.2.10.1. *Aronson et al. (1996), Siemiatycki (1991).*

B.3.2.10.1.1. ***Author's abstract.***

> A population-based case-control study of cancer and occupation was carried out in Montréal, Canada. Between 1979 and 1986, 449 pathologically confirmed cases of prostate cancer were interviewed, as well as 1,550 cancer controls and 533 population controls. Job histories were evaluated by a team of chemist/hygienists using a checklist of 294 workplace chemicals. After preliminary evaluation, 17 occupations, 11 industries, and 27 substances were selected for multivariate logistic regression analyses to estimate the odds ratio between each occupational circumstance and prostate cancer with control for potential confounders. There was moderate support for risk due to the following occupations: electrical power workers, water transport workers, aircraft fabricators, metal product fabricators, structural metal erectors, and railway transport workers. The following substances exhibited moderately strong associations: metallic dust, liquid fuel combustion products, lubricating oils and greases, and polyaromatic hydrocarbons from coal. While the population attributable risk, estimated at between 12% and 21% for these occupational exposures, may be an overestimate due to our method of analysis, even if the true attributable fraction were in the range of 5–10%, this represents an important public health issue.

B.3.2.10.1.2. *Study description and comment*. Aronson et al. (1996) and Siemiatycki (1991) reported data from a case-control study of occupational exposures and prostate cancer conducted in Montreal, Quebec (Canada) and was part of a larger study of 10 other site-specific cancers and occupational exposures. The investigators identified 557 newly diagnosed cases of prostate cancer (ICD-O, 185), confirmed on the basis of histology reports, between 1979 and 1985; 449 of these participated in the study interview (80.6% participation). One control group consisted of patients with other forms of cancer recruited through the same study procedures and time period as the prostate cancer cases. A population-based control group (n = 533, 72% response), frequency matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Aronson et al. (1996) presents observations of logistic regression analyses examining industries, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE from Mantel-Haenszel analyses are found in the original report of Siemiatycki (1991). Any exposure to TCE was 2% among cases (n = 11) and <2% for substantial TCE exposure (n = 7); "substantial" is defined as $\geq$10 years of exposure for the period up to 5 years before diagnosis.

Logistic regression models adjusted for age, education, and ethnicity (Aronson et al., 1996) or Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, coffee, and ethnic origin (Siemiatycki, 1991). Odds ratios for TCE exposure are presented with 90% confidence intervals in Siemiatycki (1991) and 95% confidence intervals in Aronson et al. (1996).

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of prostate cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Aronson KJ, Siemiatycki J, Dewar R, Gérin M. 1996. Occupational risk factors for prostate cancer: Results from a case-control study in Montréal, Canada. Am J Epidemiol 143:363−373.

Siemitycki J. 1991. Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Boca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	557 prostate cancer cases were identified among male Montreal residents between 1979 and 1985 of which 449 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of other cancer cases identified in the larger study. Participation rate: cases, 83.1%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O, 185 (Malignant neoplasm of prostate).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	449 cases (80.6% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases (*n* = 11); Substantial TCE exposure (Exposure for $\geq$10 yrs and up to 5 yrs before disease onset), <2% cases (*n* = 7).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, ethnic origin, socioeconomic status, Quetlet as an index of body mass, and respondent status (Aronson et al., 1996). Age, family income, cigarette smoking, ethnic origin, and respondent status (Siemiatycki, 1991).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Aronson et al., 1996).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.11. *Renal Cell Carcinoma Case-Control Studies—Arnsberg Region of Germany*

A series of studies (including Henschler et al. [1995], discussed in cohort study section) have been conducted in an area with a long history of trichloroethylene use in several industries. The main importance of these studies is that there is considerable detail on the nature of exposures, which made it possible to estimate the order of magnitude of exposure even though there were no direct measurements.

B.3.2.11.1. *Brüning et al. (2003).*

B.3.2.11.1.1. ***Author's abstract.***

> BACKGROUND: German studies of high exposure prevalence have been debated on the renal carcinogenicity of trichloroethylene (TRI). METHODS: A consecutive hospital-based case-control study with 134 renal cell cancer (RCC) cases and 401 controls was conducted to reevaluate the risk of TRI in this region which were estimated in a previous study. Exposure was self-assessed to compare these studies. Additionally, the job history was analyzed, using expert-based exposure information. RESULTS: The logistic regression results, adjusted for age, gender, and smoking, confirmed a TRI-related RCC risk in this region. Using the database CAREX for a comparison of industries with and without TRI exposure, a significant excess risk was estimated for the longest held job in TRI-exposing industries (odds ratio (OR) 1.80, 95% confidence interval (CI) 1.01-3.20). Any exposure in "metal degreasing" was a RCC risk factor (OR 5.57, 95% CI 2.33-13.32). Self-reported narcotic symptoms, indicative of peak exposures, were associated with an excess risk (OR 3.71, 95% CI 1.80-7.54). CONCLUSIONS: The study supports the human nephrocarcinogenicity of trichloroethylene.

B.3.2.11.1.2. *Study description and comment*. This study is a second case-control follow-up of renal cell cancer in the Arnsberg area of Germany, which was intended to deal with some of the methodological issues present in the two earlier studies. The major advantage of studies in the Arnsberg area is the high prevalence of exposure to trichloroethylene because of the large number of companies doing the same kind of industrial work. An interview questionnaire procedure for self-assessment of exposures similar to the one used by Vamvakas et al. (1998) was used to obtain detailed information about solvents used, job tasks, and working conditions, as well as the occurrence of neurological symptoms. The industry and job title information in the subjects' job histories were also analyzed by two schemes of expert-rated exposure assignments for broad groups of jobs. The CAREX database from the European Union, for industry categories, and the British JEM developed by Pannett et al. (1985), for potential exposure to chemical classes or specific chemical, but not TCE, was adopted in an attempt to obtain a potentially less biased assessment of exposures.

Exposure prevalences for employment in industries with potential TCE and perchloroethylene exposures was high in both cases (87%) and controls (79%) using the CAREX approach but much lower using the JEM approach for potential exposure to degreasing agents (12% cases, 9% controls), self-reported exposure to TCE (18% cases, 10% controls), and TCE exposure with any symptom occurrence (14% cases, 4% controls). Both the CAREX and British JEM rating approaches are very broad and they have potentially high rates of misclassification of exposure intensity in job groupings and industry groupings. In an attempt to avoid reporting biases associated with the legal proceeding for compensation, analyses were conducted on self-reported exposure to selected agents (yes or no). The regional use of trichloroethylene and perchloroethylene (tetrachloroethylene) were so widespread that most individuals recognized the local abbreviations. If individuals claimed to be exposed when they were not, it would reduce the finding of a relationship if one existed. Similarly, subjects were grouped by frequency of perceived symptoms (any, less than daily, daily) associated with TCE or perchloroethylene exposure. Overreporting would also introduce misclassification and reduce evidence of any relationship. Self-reporting of exposure to chemicals in case-control studies, generally, is considered unreliable since, within the broad population, workers rarely know specific chemicals to which they have potential exposure. However, in cohort studies and case-control studies in which one industry dominates a local population such as in this study, this is less likely because the numbers of possible industries and job titles are much smaller than in a broad population. The Arnsberg area studies focused on a small area where one type of industry was very prevalent, and that industry used primarily just two solvents: trichloroethylene and tetrachloroethylene. As a result, it was common knowledge among the workers what solvent an individual was using, and, for most, it was trichloroethylene. Self-reported TCE exposure is considered to be less biased compared to possible misclassification bias associated with using the CAREX exposure assessment approach which identified approximately 90% of all cases as holding a job in an industry using TCE or perchloroethylene (see above discussion).

Some subjects in Brüning et al. (2003) are drawn from the underlying Arnsberg population as studied by Vamvakas et al. (1998) (reviewed below) and TCE exposures to these subjects would be similar—substantial, sustained high exposures to TCE at 400−600 ppm during hot dip cleaning and greater than 100 ppm overall. However, the larger ascertainment area outside the Arnsberg region for case and control identification may have resulted in a lower exposure prevalence compared to Vamvakas et al. (1998).

Brüning T, Pesch B, Wiesenhütter B, Rabstein S, Lammert M, Baumüller A, Bolt H. 2003. Renal cell cancer risk and occupational exposure to trichloroethylene: results of a consecutive case-control study in Arnsberg, Germany. Am J Ind Med 23:274−285.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	From abstract—study aim was to "reevaluate the risk of TRI in this region which were estimated in a previous study."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	162 renal cell carcinoma cases identified from September 1999 to April 2000 and who had undergone nephrectomy between 1992 and 2000 [a time period preceding that adopted in Vamvakas et al., 1998] from a regional hospital urology department in Arnsberg, Germany; 134 of the recruited cases were interviewed. 401 hospital controls were interviewed between 1999 and 2000 from local surgery departments or geriatric departments and frequency matched to cases by sex and age. 134 of 162 (83%) cases; response rate among controls could not be calculated lacking information on the number of eligible controls.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	N/A

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Face-to-face interview with subjects or their next of kin using a structured questionnaire with questions to obtain information on a complete job history by job title, supplemental information on job tasks with suspected exposure to specific agents, medical history, and personal habits. Questionnaires also sought self-reported information on duration and frequency of exposure to TCE and perchloroethylene, and, for these individuals, frequency of narcotic symptoms as a marker of high peak exposure. Jobs titles were coded according to a British classification of occupations and industries with potential chemical-specific exposures identified for each occupation using CAREX, a carcinogen exposure database or the British job-exposure matrix of Pannett et al. (1985) for chemical groupings (e.g., degreasing agents, organic solvents).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	100% of cases or their NOK and 100% controls with face-to-face interviews.
Blinded interviewers	No information on whether interviewers were blinded.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 17% of case interviews with next-of-kin; all controls were alive at time of interview.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	<u>CAREX Job-exposure-matri</u>x 117 cases with TCE exposure (87% exposure prevalence among cases). 316 cases with TCE exposure (79% exposure prevalence among controls). <u>Self-reported TCE exposure</u> 25 cases with TCE exposure (18% exposure prevalence among cases). 38 cases with TCE exposure (9.5% exposure prevalence among controls).

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and tobacco smoking.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes, duration of exposure as 4 categories (no, <10 yrs, 10−<20 years, and 20+ yrs.
Documentation of results	Yes.

B.3.2.11.2. *Pesch et al. (2000b).*

B.3.2.11.2.1. *Author's abstract.*

> BACKGROUND: This case-control study was conducted to estimate the renal cell cancer (RCC) risk for exposure to occupation-related agents, besides other suspected risk factors. METHODS: In a population-based multicentre study, 935 incident RCC cases and 4298 controls matched for region, sex, and age were interviewed between 1991 and 1995 for their occupational history and lifestyle habits. Agent-specific exposure was expert-rated with two job-exposure matrices and a job task-exposure matrix. Conditional logistic regression was used to calculate smoking adjusted odds ratios (OR). RESULTS: Very long exposures in the chemical, rubber, and printing industries were associated with risk for RCC. Males considered as 'substantially exposed to organic solvents' showed a significant excess risk (OR = 1.6, 95% CI : 1.1-2.3). In females substantial exposure to solvents was also a significant risk factor (OR = 2.1, 95% CI : 1.0-4.4). Excess risks were shown for high exposure to cadmium (OR = 1.4, 95% CI : 1.1-1.8, in men, OR = 2.5, 95% CI : 1.2-5.3 in women), for substantial exposure to lead (OR = 1.5, 95% CI : 1.0-2.3, in men, OR = 2.6, 95% CI : 1.2-5.5, in women) and to solder fumes (OR = 1.5, 95% CI : 1.0-2.4, in men). In females, an excess risk for the task 'soldering, welding, milling' was found (OR = 3.0, 95% CI : 1.1-7.8). Exposure to paints, mineral oils, cutting fluids, benzene, polycyclic aromatic hydrocarbons, and asbestos showed an association with RCC development.
> CONCLUSIONS: Our results indicate that substantial exposure to metals and solvents may be nephrocarcinogenic. There is evidence for a gender-specific susceptibility of the kidneys.

B.3.2.11.2.2. *Study description and comment*. This multicenter study of renal cell carcinoma and bladder cancer and in Germany, which included the Arnsberg region plus four others, identified two case series from participating hospitals, 1,035 urothelial cancer cases and 935 renal cell carcinoma cases with a single population control series matched to cases by region, sex, and age (1:2 matching ratio to urothelial cancer cases and 1:4 matching ratio to renal cell carcinoma cases). A strength of the study was the high percentage of interviews with renal cell carcinoma cases within 2 months of diagnosis (88.5%), reducing bias associated with proxy or next-of-kin interview, and few cases diagnoses confirmed by sonography only (5%). In all, 935 (570 males, 365 females) renal cell carcinoma cases were interviewed face-to-face with a structured questionnaire.

Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, trichloroethylene, and tetrachloroethylene) to evaluate TCE potential using a JTEM. A category of "any use of a solvent" mixes the large number with infrequent slight contact with the few noted earlier who have high intensity and prolonged contact. Analyses examining trichloroethylene exposure using either the JEM of JTEM assigned a cumulative TCE exposure index of none to low, medium high and substantial, defined as the product of exposure probability x intensity x duration with the following cutpoints: none to low, <30th percentile of cumulative exposure scores; medium, 30th–<60th percentile; high, 60th–<90th percentile; and, substantial, $\geq$90th percentile. The use of the German JEM identified approximately twice as many cases with any potential TCE exposure (42%) compared to the JTEM (17%) and, in both cases, few cases identified with substantial exposure, 6% by JEM and 3% by JTEM. Pesch et al. (2000b) noted "exposure indices derived from an expert rating of job tasks can have a higher agent-specificity than indices derived from job titles." For this reason, the JTEM approach with consideration of job tasks is considered as a more robust exposure metric for examining TCE exposure and renal cell carcinoma due to likely reduced potential for exposure misclassification compared to TCE assignment using only job history and title.

While this case-control study includes the Arnsberg area, several other regions are included as well, where the source of the trichloroethylene and chlorinated solvent exposures are much less well defined. Few cases were identified as having substantial exposure to TCE and, as a result, most subjects identified as exposed to trichloroethylene probably had minimal contact, averaging concentrations of about 10 ppm or less (NRC, 2006).

Pesch B, Haerting J, Ranft U, Klimpet A, Oelschägel, Schill W, and the MURC Study Group. 2000b. Occupational risk factors for renal cell carcinoma: agent-specific results from a case-control study in Germany. Int J Epidemiol 29:1014−1024.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This case-control study was conducted to estimate RCC risk for exposure to occupational-related agents; chlorinated solvents including trichloroethylene were identified as exposures of *a priori* interest.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	935 RCC cases were identified from hospitals in a five-region area in Germany between 1991 and 1995. Cases were confirmed histologically (95%) or by sonography (5%) and selected without age restriction. 4,298 population controls identified from local residency registries in the five-region area were frequency matched to cases by region, sex, and age. Participation rate: cases, 88%; controls, 71%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	N/A

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	A trained interviewer interviewed subjects using a structured questionnaire which covered occupational history and job title for all jobs held longer than 1 yr, medical history, and personal information. Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, trichloroethylene, and tetrachloroethylene) and chemical-specific exposure were assigned using a JTEM. Exposure index for each subject is the sum over all jobs of duration x probability x intensity. A four category grouping was used in statistical analyses defined by exposure index distribution of controls: no-low; medium, 30th percentile; high, 60th percentile; substantial, 90th percentile.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Interviewers carried out face-to-face interview with all cases and controls. All cases were interviewed in the hospital; 88.5% of cases were interviewed within 2 mos after diagnosis. All controls had home interviews.
Blinded interviewers	No , by nature of interview location.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	JEM: 391 cases with TCE exposure index of medium or higher (42% exposure prevalence among cases). JTEM: 172 cases with TCE exposure index of medium or higher (18% exposure prevalence among cases). No information is presented in paper on control exposure prevalence.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, study center, and smoking.
Statistical methods	Conditional logistic regression.
Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.2.11.3. *Vamvakas et al. (1998).*

B.3.2.11.3.1. *Author's abstract.*

> A previous cohort-study in a cardboard factory demonstrated that high and prolonged occupational exposure to trichloroethene (C2HCl3) is associated with an increased incidence of renal cell cancer. The present hospital-based case/control study investigates occupational exposure in 58 patients with renal cell cancer with special emphasis on C2HCl3 and the structurally and toxicologically closely related compound tetrachloroethene (C2Cl4). A group of 84 patients from the accident wards of three general hospitals in the same area served as controls. Of the 58 cases, 19 had histories of occupational C2HCl3 exposure for at least 2 years and none had been exposed to C2Cl4; of the 84 controls, 5 had been occupationally exposed to C2HCl3 and 2 to C2Cl4. After adjustment for other risk factors, such as age, obesity, high blood pressure, smoking and chronic intake of diuretics, the study demonstrates an association of renal cell cancer with long-term exposure to C2HCl3 (odds ratio 10.80; 95% CI: 3.36-34.75).

B.3.2.11.3.2. *Study description and comment*. In a follow-up to Henschler et al. (1995) (discussed below), a case-control study was conducted in the Arnsberg region of Germany where there has long been a high prevalence of small enterprises manufacturing small metal parts and goods, such as nuts, lamps, screws, and bolts. Both cases and controls were identified from hospital records; cases from of a large regional hospital in North Rhine Wetphalia during the period 1987 and 1992 and controls who were admitted to accident wards during 1993 at three other regional hospitals. Control selection was carried out independent of cases demographic risk factors, i.e., controls were not matched to cases. Controls may not be fully representative of the case series (NRC, 2006); they were selected from a time period after case selection which may introduce bias if TCE use changes over time resulted in decreased potential for exposure among controls, and use of accident ward patients may be representative of the target population.

Exposures to TCE resulted from dipping metal pieces into vats, with room temperatures up to 60°C, and placing the wet parts on tables to dry. Some work rooms were noted to be small and poorly ventilated. These conditions are likely to result in high inhalation exposure to trichloroethylene (100−500 ppm). Cherrie et al. (2001) estimated the long-term exposures to be approximately 100 ppm. Some of the cases included in this study were also pending legal compensation. As a result, there had been considerable investigation of the exposure situation by occupational hygienists from the Employer's Liability Insurance Association and occupational physicians, including walk-through visits and interviews of long-term employees. The legal action could introduce a bias, a tendency to overreport some of the subjective reports by the subjects. However, the objective working conditions were assessed by knowledgeable professionals, who corroborated the presence of the poorly controlled hot dip tanks, extensive use of trichloroethylene for all types of cleaning, and the process descriptions.

NRC (2006) discussed a number of criticisms in the literature on Vamvakas et al. (1998) by Green and Lash (1999), Cherrie et al. (2001), and Mandel (2001) and noted the direction of possible bias would be positive or negative depending on the specific criticism. Overall, cases in this study substantial, sustained exposures to high concentrations of trichloroethylene at 400−600 ppm during hot dip cleaning and greater than 100 ppm overall and observations can inform hazard identification although the magnitude of observed association is uncertain give possible biases.

Vamvakas S, Brüning T, Thomasson B, Lammert M, Baumüller A, Bolt HM, Dekant W, Birner G, Henschler D, Ulm K. 1998. Renal cell cancer risk and occupational exposure to trichloroethylene: results of a consecutive case-control study in Arnsberg, Germany. Am J Ind Med 23:274−285.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes. From introduction—study aim was designed to investigate further the role of occupation exposure to TCE/perchloroethylene in the formation of renal cancer.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	73 renal cell carcinoma cases that had undergone nephrectomy between December 1987 and May 1992 from a hospital urology department in Arnsberg, Germany were contacted by mail; 58 of the recruited cases were. 112 controls identified from accident wards of three area hospitals were interviewed during 1993. Controls underwent abdominal sonography to exclude kidney cancer. 62 of 73 (85%) cases and 84 of 112 (75%) of controls participated in study.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	N/A

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Face-to-face interview with subjects or, if deceased, with their next of kin or former colleagues using a structured questionnaire with questions to obtain information on job tasks with selected exposure to specific agents and to self-reported selected exposures. A supplemental questionnaire on job conditions was administered to subjects reporting exposure to TCE and perchloroethylene. Subjects with TCE exposures were primarily exposed through degreasing operations in small businesses. Self-reported TCE exposure was ranked using a semiquantitative scale based upon total exposure time and frequency/duration of self-reported acute prenarcotic symptoms. **Cherrie et al. (2001) estimated that the machine cleaning exposures to trichloroethylene were ~400−600 ppm, with long-term average TCE exposure as ~100 ppm.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Personal physicians interviewed 100% of cases or their NOK/former colleague and 100% controls.
Blinded interviewers	Interviewers were not blinded nor was developments of exposure assessment semiquantitative scale.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No information provided in paper on number of cases with NOK interviews or interviews with former colleagues; all controls were alive and interviewed by their personal physician.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	19 cases with TCE or perchloroethylene exposure (33% exposure prevalence) and 1 control with perchloroethylene exposure.

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, obesity, high blood pressure, smoking, and diuretic use.
Statistical methods	Mantel-Haenszel χ^2.
Exposure-response analysis presented in published paper	Yes, semiquantitative scale of 4 categories (no, +, ++, +++).
Documentation of results	No information on number of eligible controls or number interviews with case NOK or former colleagues.

B.3.2.12. *Renal Cell Carcinoma Case-Control Studies—Arve Valley Region of France*

A case-control study was conducted in the Arve Valley to examine the *a priori* hypothesis of an association with renal cell carcinoma and trichloroethylene exposure. The Arve Valley, like the Arnsburg Region in Germany, has a long history of trichloroethylene use in the screw-cutting industry. The Arve Valley, situated in the Rhône-Alpes region of eastern France is a major metalworking sector with around 800 small and medium-sized firms specializing in "screw-cutting" or the machining of small mechanical parts from bars, in small, medium, and large series on conventional automatic lathes or by digital control. This industry evolved around the time of World War I from the region's expertise in clock-making. A major point of this study is that it was designed as a follow-up study to the German renal cell cancer case-control studies but in a different population with similar exposure patterns and with high prevalence of exposure to trichloroethylene. For this reason, there is considerable detail on the nature of exposure, which made it possible to estimate the order of magnitude of exposure, even though there were not direct measurements.

B.3.2.12.1. *Charbotel et al. (2009), Charbotel et al. (2007) Charbotel et al. (2006).*

B.3.2.12.1.1. *Charbotel et al. (2009) abstract.*

> *Abstract Background*– Several studies have investigated the association between trichloroethylene (TCE) exposure and renal cell cancer (RCC) but findings were inconsistent. The analysis of a case control study has shown an increased risk of RCC among subjects exposed to high cumulative exposure. The aim of this complementary analysis is to assess the relevance of current exposure limits regarding a potential carcinogenic effect of TCE on kidney.
> *Methods*– Eighty-six cases and 316 controls matched for age and gender were included in the study. Successive jobs and working circumstances were described using a detailed occupational questionnaire. An average level of exposure to TCE was attributed to each job period in turn. The main occupational exposures described in the literature as increasing the risk of RCC were assessed as well as non-occupational factors. A conditional logistic regression was performed to test the association between TCE and RCC risk. Three exposure levels were studied (average exposure during the eight-hour shift): 35 ppm, 50 ppm and 75 ppm. Potential confounding factors identified were taken into account at the threshold limit of 10% ($p = 0.10$) (body mass index [BMI], tobacco smoking, occupational exposures to cutting fluids and to other oils).
> *Results*– Adjusted for tobacco smoking and BMI, the odd-ratios associated with exposure to TCE were respectively 1.62 [0.77–3.42], 2.80 [1.12–7.03] and 2.92 [0.85–10.09] at the thresholds of 35 ppm, 50 ppm and 75 ppm. Among subjects exposed to cutting fluids and TCE over 50 ppm, the OR adjusted for BMI, tobacco smoking and exposure to other oils was 2.70 [1.02–7.17].

Conclusion– Results from the present study as well as those provided in the international literature suggest that current French occupational exposure limits for TCE are too high regarding a possible risk of RCC.

B.3.2.12.1.2. *Charbotel et al. (2007) abstract.*

Background: We investigated the association between exposure to trichloroethylene (TCE) and mutations in the von Hippel-Lindau (VHL) gene and the subsequent risk for renal cell carcinoma (RCC).
Methods: Cases were recruited from a case-control study previously carried out in France that suggested an association between exposures to high levels of TCE and increased risk of RCC. From 87 cases of RCC recruited for the epidemiological study, 69 were included in the present study. All samples were evaluated by a pathologist in order to identify the histological subtype and then be able to focus on clear cell RCC. The majority of the tumor samples were fixed either in formalin or Bouin's solutions. The majority of the tumors were of the clear cell RCC subtype (48 including 2 cystic RCC). Mutation screening of the 3 VHL coding exons was carried out. A descriptive analysis was performed to compare exposed and non exposed cases of clear cell RCC in terms of prevalence of mutations in both groups.
Results: In the 48 cases of RCC, four VHL mutations were detected: within exon 1 (c.332G>A, p.Ser111Asn), at the exon 2 splice site (c.463+1G>C and c.463+2T>C) and within exon 3 (c.506T>C, p.Leu169Pro). No difference was observed regarding the frequency of mutations in exposed versus unexposed groups: among the clear cell RCC, 25 had been exposed to TCE and 23 had no history of occupational exposure to TCE. Two patients with a mutation were identified in each group.
Conclusion: This study does not confirm the association between the number and type of VHL gene mutations and exposure to TCE previously described.

B.3.2.12.1.3. *Charbotel et al. (2006) abstract.*

Background: We investigated the association between exposure to trichloroethylene (TCE) and mutations in the von Hippel-Lindau (VHL) gene and the subsequent risk for renal cell carcinoma (RCC).
Methods: Cases were recruited from a case-control study previously carried out in France that suggested an association between exposures to high levels of TCE and increased risk of RCC. From 87 cases of RCC recruited for the epidemiological study, 69 were included in the present study. All samples were evaluated by a pathologist in order to identify the histological subtype and then be able to focus on clear cell RCC. The majority of the tumor samples were fixed either in formalin or Bouin's solutions. The majority of the tumors were of the clear cell RCC subtype (48 including 2 cystic RCC). Mutation screening of the 3 VHL coding exons was carried out. A descriptive analysis was performed to compare

> exposed and non-exposed cases of clear cell RCC in terms of prevalence of mutations in both groups.
> Results: In the 48 cases of RCC, four VHL mutations were detected: within exon 1 (c.332G>A, p.Ser111Asn), at the exon 2 splice site (c.463+1G>C and c.463+2T>C) and within exon 3 (c.506T>C, p.Leu169Pro). No difference was observed regarding the frequency of mutations in exposed versus unexposed groups: among the clear cell RCC, 25 had been exposed to TCE and 23 had no history of occupational exposure to TCE. Two patients with a mutation were identified in each group.
> Conclusion: This study does not confirm the association between the number and type of VHL gene mutations and exposure to TCE previously described.
>
> To test the effect of the exposure to trichloroethylene (TCE) on renal cell cancer (RCC) risk, a case–control study was performed in the Arve Valley (France), a geographic area with a high frequency and a high degree of such exposure. Cases and controls were selected from various sources: local general practitioners and urologists practicing in the area and physicians (urologists and oncologists) from other hospitals of the region who might treat patients from this area. Blinded telephone interviews with cases and controls were administered by a single trained interviewer using occupational and medical questionnaires. The analysis concerned 86 cases and 316 controls matched for age and gender. Three approaches were developed to assess the link between TCE exposure and RCC: exposure to TCE for at least one job period (minimum 1 year), cumulative dose number of ppm of TCE per job period multiplied by the number of years in the job period) and the effect of exposure to peaks. Multivariate analysis was performed taking into account potential confounding factors. Allowing for tobacco smoking and Body Mass Index, a significantly 2-fold increased risk was identified for high cumulative doses: odds ratio (OR) = 2.16 (1.02–4.60). A dose-response relationship was identified, as was a peak effect; the adjusted OR for highest class of exposure-plus-peak being 2.73 (1.06–7.07). After adjusting for exposure to cutting fluids the ORs, although still high, were not significant because of lack of power. This study suggests an association between exposures to high levels of TCE and increased risk of RCC. Further epidemiological studies are necessary to analyze the effect of lower levels of exposure.

B.3.2.12.1.4. ***Study description and comment***. Cases in the population-based case-control study were obtained retrospectively from regional medical practitioners or from teaching hospitals from 1993 to 2002, and prospectively from 2002 to mid-2003. One case was excluded from analysis because it was not possible to find a control subject. Controls were either selected from the same urology practice as cases or, for cases selected from teaching hospitals, from among patients of the case's general practitioner. Telephone interviews of 87 renal cell carcinoma cases and 316 controls matched for age and sex by a trained interviewer were used to obtain information on occupational and medical history for the case-control analysis of Charbotel et al.

(2006). Of the 87 RCC cases, 67 cases provided consent for mutational analysis of which 48 cases were diagnosed with clear cell RCC, suitable for mutational analysis of the von Hippel Lindau (VHL) gene (Charbotel et al., 2007). Tissue samples were paraffin-embedded or frozen tissues and ability to fully sequence the VHL gene depended on type of the fixative procedure; only 26 clear cell RCC cases (34% of 73 clear cell RCC cases in the case-control study) could full sequencing of the VHL gene occur.

Two occupational questionnaires were administered to both cases and controls, a questionnaire developed specifically to evaluate jobs and exposure potential in the screw-cutting industry and a more general one for any other jobs. Interviewers were essentially blinded to subject status as case or control for the occupational questionnaires given the medical questionnaire was administered afterwards (Fevotte et al., 2006). The medical questionnaire included familial kidney disease and medical history, body mass index, and history of smoking. A task/TCE-Exposure Matrix was designed using information obtained from questionnaires and routine atmospheric monitoring of work shops or biological monitoring (U-TCA) of workers carried out since the 1960s. Questionnaires were used to elicit from each subject the main tasks associated with each job, working conditions, activities or jobs that might involve TCE exposures and possible exposure to other occupational risk factors for renal cell carcinoma.

The JEM linked to corresponding TCE-exposure levels using available industrial hygiene monitoring data on atmospheric TCE levels and from biological measurement on workers. Estimates reflected task duration, use of protective equipment and distance from TCE source, as well, as both dermal and inhalation exposure routes. Estimated TCE intensities for jobs associated with open cold degreasing were 15−18 ppm, 120 ppm for jobs working near open hot degreasing machines, with up to 300 ppm for work directly above tank and for job and intensities of 300 to 600 ppm for emptying, cleaning and refilling degreasers. Eight local physicians with knowledge of working conditions corroborated the working conditions for individual job periods after 1980 in screw-cutting shops. Overall, there was good agreement (72%) between physician and the JEM. Three exposure surrogates were assigned to each case and control: time-weighted-average exposure (Charbotel et al., 2009), cumulative exposure (Charbotel et al., 2006), and cumulative exposure with and without peak exposure (Charbotel et al., 2006).

An 8-hour time-weighted average (TWA) exposure concentration was developed for each job period from 1924 to 2003 and was the product of the task-specific estimated TCE intensity and duration of task. A subject's lifetime 8-hour TWA was the sum of each job period specific estimated TWA. Exposure peak, daily exposure reaching $\geq$200 ppm for at least 15 minutes, was assessed as an additive factor and was defined by frequency (seldom exposed, few times yearly to frequently exposure, few time weekly).

Over the study period, 19% (295 of 1,486) job periods were assessed as having TCE exposure with an 8-hour TWA of less than 35 ppm for 72% of exposed jobs and >75 ppm for 5% of exposed jobs. Exposure prevalence to TCE peaked in the 1970s with roughly 20% of job periods with TCE exposure and 8% of subjects identified with >75 ppm. By the 1990s, exposure prevalence had not only decreased to 7% but also exposure intensity, only 5% of job periods with >75 ppm.

Cumulative TCE exposure was the sum of 8-hour TWAs over all job periods with statistical analysis using four categories: no, low, medium, and high. These were defined as low, 5−150 ppm-years; medium, 155−335 ppm-year; and high, >335 ppm-years (HSIA, 2005). Analyses were also carried out examining peak exposure, classified as yes/no and without assignment of quantitative level, as additional exposure to average TCE concentration; 33 subjects were exposed to peaks and very few to high peaks.

The high exposure prevalence and strong approach for exposure assessment provides Charbotel et al. (2006, 2009) more statistical power and ability to assess association of renal cell carcinoma and TCE exposure. However, the low participation rate, inability to fully sequence the VHL gene in all clear cell RCC cases, the lower background prevalence of mutations (15% in this study compared to roughly 50% in other series) in Charbotel et al. (2007) suggest a relative insensitivity of assay used and lack of a positive control limits the mutational analysis. These methodological limitations introduce bias with greater uncertainties for evaluating consistency of findings with somatic VHL mutations observed in other TCE-exposed RCC cases (Brauch et al., 1999; Brüning et al., 1997). TCE exposure prevalence (>5 ppm-year) in Charbotel et al. (2006) was 43% among cases and is higher than that observed in other population-based case-control studies of renal cell carcinoma and TCE (e.g., Pesch et al., 2000a). While some subjects had jobs with exposures to high concentrations of TCE during the 1970s and 1980s, a large percentage of jobs were to TCE concentrations of less than 35 ppm (8-hour TWA). Jobs with high TCE concentrations also were identified as having frequent exposure to peak TCE concentrations, particularly before 1980. Peak TCE estimates in this study were judged to be lower than those in German studies of the Arnsberg region (Henschler et al., 1995; Vamvakas et al., 1998) but judged higher than those of Hill Air Force Base civilian workers (Blair et al., 1998; Stewart et al., 1991) due to a lower frequency of degreasing tasks in Blair et al. (1998) cohort and to slower technological changes in degreasing process in the French case-control study (Fevotte et al., 2006).

Charbotel B, Fevotte J, martin JL, Bergeret A. 2009. Cancer du rein et expositions au trichloroethylene: les valeurs limites d'exposition professionnelle fraçaises en vigueur sont-elles adaptées. Rev Epidemiol Sante Publique 57:41-47.

Charbotel B, Fevotte J, Hours M, Martin J-L, Bergeret A. 2006. Case-control study on renal cell cancer and occupational exposure to trichloroethylene. Part II: Epidemiological Aspects. Ann Occup Hyg 50:777−787.

Fevotte J, Charbotel B, Muller-Beaute P, Martin J-L, Hours, Bergeret A. 2006. Case-control study on renal cell cancer and occupational exposure to trichloroethylene. Part I: Exposure assessment. Ann Occup Hyg 50:765−775.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes. From abstract—study aim was to "test the effect of TCE exposure on renal cell cancer."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	117 cases of renal cell carcinoma patients were identified retrospectively from 1993 to June 2002, and prospectively from June 2002 to June 2003 from patients of urology practices and hospital urology and oncology departments in the region of Arve Valley, France. 404 controls were identified from the same urology practice or from the same general practitioner, for cases identified from hospital records and matched on residency in the geographic study area at time of case diagnosis, sex, and year of birth. Controls sought medical treatment for conditions other than kidney or bladder cancer. Case definition included clear cell and other subtypes of renal cell carcinoma including chromophil, chromophobe and collecting duct carcinomas. 87 or 117 (74%) cases and 316 of 404 (78%) controls participated in study.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	N/A

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Occupational questionnaires sought information for each study subject a complete job history and was followed-up with either a questionnaire specific for jobs and exposures in the screw-cutting industry or a General Occupational Questionnaire, which ever was more applicable to subject. Questionnaires also sought self-reported information on potential TCE exposures. A medical questionnaire seeking information on medical history and familial kidney disease was administered after occupational questionnaires. Jobs titles were coded according to standardized classification of occupations and 1,486 job periods grouped into 3 categories (screw-cutting, nonscrew-cutting but job with possible TCE exposure, and no TCE exposure). An estimated 8-hour TWA was assigned to each job and job period using a job-task-exposure matrix. RCC and TCE was examined using three exposure approaches: exposure to at least 5 ppm for at least one job period (minimum 1 yr), cumulative dose or ∑ (TCE ppm per job × years) using quantitative ranking levels (no exposure, low, medium, and high), and potential for peak defined as any exposure 200+ ppm. **TCE concentrations associated with quantitative ranking are low, 5−150 ppm-yrs; medium, 155−335 ppm-yrs; high, >335 ppm-yrs.**
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Telephone interviews were conducted by a trained interviewer.
Blinded interviewers	The paper notes interviewers were blinded "as far as possible" since medical questionnaire was administered after the occupational questionnaires.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 22% of cases were dead at time of interview compared to 7% of controls.

CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	37 cases with TCE exposure (43% exposure prevalence), 110 controls with TCE exposure (35% exposure prevalence). 16 cases with high level confidence TCE exposure (27% exposure prevalence), 37 controls with high level confidence TCE exposure (16%).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, tobacco smoking and body mass index (Charbotel et al., 2006). Age, sex tobacco smoking, body mass index, and exposure to cutting or petroleum oils (Charbotel et al., 2009).
Statistical methods	Conditional logistic regression on matched pairs.
Exposure-response analysis presented in published paper	Yes, cumulative exposure as 4 categories (no, low, medium and high exposure) and cumulative exposure plus peaks.
Documentation of results	Yes.

B.3.2.13. *Renal Cell Carcinoma Case-Control Studies in Other Regions*

B.3.2.13.1. *Parent et al. (2000b), Siemiatycki (1991).*

B.3.2.13.1.1. ***Author's abstract.***

> BACKGROUND: Little is known about the role of workplace exposures on the risk of renal cell cancer. METHODS: A population-based case-control study was undertaken in Montreal to assess the association between hundreds of occupational circumstances and several cancer sites, including the kidney. A total of 142 male patients with pathologically confirmed renal cell carcinoma, 1900 controls with cancer at other sites and 533 population-based controls were interviewed. Detailed job histories and relevant data on potential confounders were obtained. A group of chemists-hygienists evaluated each job reported and translated them into a history of occupational exposures using a checklist of 294 substances. Multivariate logistic regression models using either population, cancer controls, or a pool of both groups were used to estimate odds ratios. RESULTS: There were some indications of excess risks among printers, nursery workers (gardening), aircraft mechanics, farmers, and horticulturists, as well as in the following industries: printing-related services, defense services, wholesale trade, and retail trade. Notwithstanding the low precision of many of the odds ratio estimates, the following workplace exposures showed some evidence of excess risk: chromium compounds, chromium (VI) compounds, inorganic acid solutions, styrene-butadiene rubber, ozone, hydrogen sulphide, ultraviolet radiation, hair dust, felt dust, jet fuel engine emissions, jet fuel, aviation gasoline, phosphoric acid and inks. CONCLUSIONS: For most of these associations there exist no, or very little, previous data. Some associations provide suggestive evidence for further studies.

B.3.2.13.1.2. ***Study description and comment***. This population case-control study of histologically-confirmed kidney cancer among males who resided in the Montreal Metropolitan area relies on the use of expert assessment of occupational information on a detailed questionnaire and face-to-face interview and was part of a larger study of 10 other site-specific cancers and occupational exposures (Parent et al., 2000b; Siemiatycki, 1991). Interviewers were unblinded, although exposure assignment was carried out blinded as to case and control status. The questionnaire sought information on the subject's complete job history and included questions about the specific job of the employee and work environment. Occupations considered with possible TCE exposure included machinists, aircraft mechanics, and industrial equipment mechanics. An additional specialized questionnaire was developed for certain job title of a prior interest that sought more detailed information on tasks and possible exposures. For example, the supplemental questionnaire for machinists included a question on TCE usage. A team of industrial hygienists and chemicals assigned exposures blinded based on job title and other information obtained by questionnaire. A semiquantitative scale was developed for 300 exposures and included TCE (any, substantial). Parent et al. (2000b) presents observations of analyses examining job title, occupation, and some chemical-specific exposures, but not TCE. Observations on TCE are found in the original report of Siemiatycki (1991). Any exposure to TCE was 3% among cases but <1% for substantial TCE exposure; "substantial" is defined as >10 years of exposure for the period up to 5 years before diagnosis. The TCE exposure frequencies in this study are lower than those in Brüning et al. (2003) and Charbotel et al. (2006), studies conducted in geographical areas with a high prevalence of industries using TCE. The expert assessment method is considered a valid and reliable approach for assessing occupational exposure in community-base studies and likely less biased from exposure misclassification than exposure assessment based solely on self-reported information (IOM, 2003; Fritschi et al., 2003; Siemiatycki et al., 1997). For example, Dewar et al. (1991) examine sensitivity of JEM of Siemiatycki et al. (1987) to exposure assessment by chemists and industrial hygienists using interview information and evaluation of job histories. Specific solvents are not examined, although, a sensitive 84% and specificity of 97% was found for the JEM for general solvent exposure.

This population study of several cancer sites included histologically-confirmed cases of kidney cancer (ICD-O 189, malignant neoplasm of kidney and other and unspecified urinary organs) ascertained from 16 Montreal-area hospitals between 1979 and 1985. A total of 227 eligible kidney cancer cases were identified were identified from 19 Montreal-area hospitals; 177 cases participated in the study (78% response). One control group (n = 1,295) consisted of patients with other forms of cancer (excluding lung cancer and other intestinal cancers) recruited through the same study procedures and time period as the rectal cancer cases. A population-based control group (n = 533), frequency matched by age strata, was drawn using electoral lists and random digit dialing. All controls were interviewed using face-to-face methods; however, 20 % of the all cancer cases in the larger study were either too ill to interview or had died and, for these cases, occupational information was provided by a proxy respondent. The quality of interview conducted with proxy respondents was much lower, increasing the potential for misclassification bias, than that with the subject. The direction of this bias would diminish observed risk towards the null.

Statistical analysis are considered valid; logistic regression model which included terms for respondent status, age, smoking and body mass index in Parent et al. (2000b) and Mantel-Haenszel χ^2 stratified on age, family income, cigarette smoking, and ethic origin in Siemiatycki (1991). Odds ratios are presented with 90% confidence intervals in Siemiatycki (1991) and 95% confidence intervals in Parent et al. (2000b).

Overall, exposure assessment in this study adopted a superior approach, using expert knowledge and use of a job-exposure matrix. However, examination of NHL and TCE exposure is limited by statistical power considerations related to low exposure prevalence, particularly for "substantial" exposure. For the exposure prevalence found in this study to TCE and for kidney cancer, the minimum detectable odds ratio was 3.0 when $\beta = 0.02$ and $\alpha = 0.05$ (one-sided). The low statistical power to detect a doubling of risk and an increased possibility of misclassification bias associated with case occupational histories resulting from proxy respondents suggests a decreased sensitivity in this study for examining kidney cancer and TCE.

Parent M-E, Hua Y, Siemiatycki J. 2000b. Occupational risk factors for renal cell carcinoma in Montreal. Am J Ind Med 38:609−618.

Siemiatycki J. 1991. Risk Factors for Cancer in the Workplace. Baca Raton: CRC Press.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	277 kidney cancer cases were identified among male Montreal residents between 1979 and 1985 of which 177 (147 renal cell carcinomas) were interviewed. 740 male population controls were identified from the same source population using random digit dialing; 533 were interviewed. A second control series consisted of all other cancer controls excluding lung and bladder cancer cases. Participation rate: cases, 78%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD 189 (Malignant neoplasm of the kidney and other and unspecified urinary organs) (Siemiatycki, 1991). ICD 189.0, renal cell carcinoma (Parent et al., 2000b).
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 300 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	100% of cases and controls were interviewed face-to-face by a trained interviewer. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 16% of cases, 13% of population controls, and 22% of cancer controls had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	177 cases (78% response), 533 population controls (72%). Exposure prevalence: Any TCE exposure, 2% cases; Substantial TCE exposure (Exposure for $\geq$10 yrs and up to 5 yrs before disease onset), 1% cases.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, income, index for cigarette smoking (Siemiatycki, 1991). Age, smoking, body mass index, and proxy status (Parent et al., 2000b).
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991). Logistic regression (Parent et al., 2000b).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.13.2. *Dosemeci et al. (1999).*

B.3.2.13.2.1. ***Author's abstract.***

> BACKGROUND: Organic solvents have been associated with renal cell cancer; however, the risk by gender and type of solvents is nuclear. METHODS: We evaluated the risk of renal cell carcinoma among men and women exposed to all organic solvents-combined, all chlorinated aliphatic hydrocarbons (CAHC)-combined, and nine individual CAHC using *a priori* job exposure matrices developed by NCI in a population-based case-control study in Minnesota, U.S. We interviewed 438 renal cell cancer cases (273 men and 165 women) and 687 controls (462 men and 225 women). RESULTS: Overall, 34% of male cases and 21% of female cases were exposed to organic solvents in general. The risk of renal cell carcinoma was significantly elevated among women exposed to all organic solvents combined (OR = 2.3; 95% CI = 1.3-4.2), to CAHC combined (OR = 2.1; 95% CI = 1.1-3.9), and to trichloroethylene (TCE) (OR = 2.0; 95% CI = 1.0-4.0). Among men, no significant excess risk was observed among men exposed to any of these nine individual CAHCs, all CAHCs-combined, or all organic solvents-combined. DISCUSSION: These observed gender differences in risk of renal cell carcinoma in relation to exposure to organic solvents may be explained by chance based on small numbers, or by the differences in body fat content, metabolic activity, the rate of elimination of xenobiotics from the body, or by differences in the level of exposure between men and women, even though they have the same job title.

B.3.2.13.2.2. *Study description and comment*. Dosemeci et al. (1999) reported data from a population-based case-control study of the association between occupation exposures and renal cancer risk. The investigators identified newly diagnosed patients with histologically confirmed renal cell carcinoma from the Minnesota Cancer Surveillance System from July 1, 1988 to December 31, 1990. The study was limited to white cases, and age and gender-stratified controls were ascertained using random digit dialing (for subjects ages 20−64) and from Medicare records (for subjects 65−85 years). Of the 796 cases and 796 controls initially identified, 438 cases (273 men, 165 women) and 687 controls (462 men, 225 women) with complete personal interviews were included in the occupational analysis.

Data were obtained using in-person interviews that included demographic variables, residential history, diet, smoking habits, medical history, and drug use. The occupational history included information about the most recent and usual industry and occupation (coded using the standard industrial and occupation codes, Department of Commerce), job activities, hire and termination dates, and full/part time status. A job exposure matrix developed by the National Cancer Institute (Gomez et al., 1994) was used with the coded job data assign occupational exposure potential for 10 chlorinated aromatic hydrocarbons and organic solvents, and includes trichloroethylene.

Dosemeci et al. (1999) adopted logistic regression methods to evaluate renal cancer and occupational exposures. Odds ratios were adjusted for age, smoking, hypertension, and use of drugs for hypertension, and body mass index.

Strengths of this study include the use of incident cases of renal cancer from a defined population area, with confirmation of the diagnosis using histology reports. The occupation history was based on usual and most recent job, in combination with a relatively focused job exposure matrix. In contrast to the type of exposure assessment that can be conducted in cohort studies within a specific workplace, however, exposure measurements, based on personal or workplace measurement, were not used, and a full lifetime job history was not obtained.

Dosemeci M, Cocco P, Chow W-H. 1999. Gender differences in risk of renal cell carcinoma and occupational exposures to chlorinated aliphatic hydrocarbons. Am J Ind Med 36:54−59.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes. From abstract—study aim was to evaluate effect of organic solvents on RCC risk using *a priori* job exposure matrices.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	796 white males and females identified through the Minnesota Cancer Surveillance System with histological confirmed RCC between July 1, 1988 and December 31, 1990. Interviews were obtained for 690 subjects of which 241 were with next-of-kin and excluded; 438 cases (273 males and 165 females) were included in analysis. 707 white population controls identified through random digit dialing, and matched to cases, aged 20−65 yrs old, by age and sex using a stratified random sample or, for cases aged 65−85, from Health Care Financing Administration list. 687 controls (462 males and 225 females) are included in the analysis. Participation rate: cases, 87%; controls, 86%. Occupational analysis: cases, 55%, controls 83%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	N/A

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	A trained interviewer blinded to case and control status interviewed subjects at home using a questionnaire which covered occupational, residential, and medical histories; demographic information; and personal information. Occupational history included self-reporting of the most recent job and usual occupation and industry, employment dates, and focused on 13 specific occupations or industries. Occupation and industry were coded according to a standard occupational classification or standard industrial classification with potential chemical-specific exposures to TCE and eight other chlorinated hydrocarbons identified using the job exposure matrix of Dosemeci et al. (1994) and Gomez et al. (1994).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	All cases and controls had face-to-face interviews.
Blinded interviewers	Yes, interviewers were blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	No, subjects with next-of-kin interviews were excluded from the analysis.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancers in incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	55 cases with TCE exposure (13% exposure prevalence among cases). 69 controls cases with TCE exposure (10% exposure prevalence among controls).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, smoking, body mass index, and hypertension/ use of diuretics/use of anti-hypertension drugs.
Statistical methods	Logistic regression.

Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.2.14. *Other Cancer Site Case-Control Studies*

B.3.2.14.1. *Siemiatycki (1991), Siemiatycki et al. (1987).*

B.3.2.14.1.1. ***Author's abstract.***

> A multi-cancer site, multi-factor, case-referent study was undertaken to generate hypotheses about possible occupational carcinogens. About 20 types of cancer were included. Incident cases among men aged 35-70 years and diagnosed in any of the major Montreal hospitals were eligible. Probing interviews were carried out for 3,726 eligible cases. The interview was designed to obtain detailed lifetime job histories and information on potential confounders. Each job history was reviewed by a team of chemists who translated it into a history of occupational exposures. These occupational exposures were then analyzed as potential risk factors in relation to the sites of cancer included. For each site of cancer analyzed, referents were selected from among the other sites in the study. The analysis was carried out in stages. First a Mantel-Haenszel analysis was undertaken of all cancer-substance associations, stratifying on a limited number of covariates, and, then, for those associations which were noteworthy in the initial analysis, a logistic regression analysis was made taking into account all potential confounders. This report describes the fieldwork and analytical methods.

B.3.2.14.1.2. ***Study description and comment***. Siemiatycki (1991) reported data from a case-control study of occupational exposures and several site-specific cancers, including lung and pancreas, conducted in Montreal, Quebec (Canada). Other cases included in this study were cancers of the bladder, colon, rectum, esophagus prostate, and lymphatic system (NHL); a description of the other case series are found in other sections in this appendix. The investigators identified 1,082 newly diagnosed cases of lung cancer (ICD-O, 162) and 165 newly diagnosed cases of pancreatic cancer (ICD-O, 157), confirmed on the basis of histology reports, between 1979 and 1985; 857 lung cancer (79.2%) and 117 pancreatic cancer cases (70.7%) participated in the study interview. One control group consisted of patients with other forms of cancer recruited through the same study procedures and time period as the melanoma cancer cases. The control series for lung cancer cases excluded other lung cancer cases; the control series for pancreatic cancer cases excluded all lung cancer cases. Additionally, a population-based control group (n = 533, 72% response), frequency matched by age strata, was drawn using electoral lists and random digit dialing. Face-to-face interviews were carried out with 82% of all cancer cases with telephone interview (10%) or mailed questionnaire (8%) for the remaining cases. Twenty percent of all case interviews were provided by proxy respondents. The occupational assessment consisted of a detailed description of each job held during the working lifetime, including the company, products, nature of work at site, job activities, and any additional information that could furnish clues about exposure from the interviews.

A team of industrial hygienists and chemists blinded to subject's disease status translated jobs into potential exposure to 294 substances with three dimensions (degree of confidence that exposure occurred, frequency of exposure, and concentration of exposure). Each of these exposure dimensions was categorized into none, any, or substantial exposure. Any exposure to TCE was 2% among cases (*n* = 21 lung cancer cases, 2 pancreatic cancer cases) and 1% for substantial TCE exposure (*n* = 9 lung cancer cases); "substantial" is defined as $\geq$10 years of exposure for the period up to 5 years before diagnosis. None of the pancreatic cancer cases was identified with "substantial" exposure to TCE.

Mantel-Haenszel χ^2 analyses examined occupation exposures and lung cancer stratified on age, family income, cigarette smoking, ethnic origin, alcohol consumption, and respondent status or pancreatic cancer stratified on age, income, cigarette smoking, and respondent status (Siemiatycki, 1991). Odds ratios for TCE exposure in Siemiatycki (1991) are presented with 90% confidence intervals.

The strengths of this study were the large number of incident cases, specific information about job duties for all jobs held, and a definitive diagnosis of cancer. However, the use of the general population (rather than a known cohort of exposed workers) reduced the likelihood that subjects were exposed to TCE, resulting in relatively low statistical power for the analysis. The job exposure matrix, applied to the job information, was very broad since it was used to evaluate 294 chemicals.

Siemitycki J. 1991. Risk Factors for Cancer in the Workplace. J Siemiatycki, Ed. Boca Raton: CRC Press.

Siemiatycki J, Wacholder S, Richardson L, Dewar R, Gérin M. 1987. Discovering carcinogens in the occupational environment. Scand J Work Environ Health 13:486−492.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This population case-control study was designed to generate hypotheses on possible association between 11 site-specific cancers and occupational title or chemical exposures.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	1,082 lung cases were identified among male Montreal residents between 1979 and 1985 of which 857 were interviewed; 165 cases were identified among male Montreal residents between 1979 and 1985 of which 117 were interviewed. 740 eligible male controls identified from the same source population using random digit dialing or electoral lists; 533 were interviewed. A second control series consisted of other cancer cases identified in the larger study. Participation rate: lung cancer cases, 79.2 %, pancreatic cancer cases, 70.7%; population controls, 72%.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O, 122 (Malignant neoplasm of trachea, bronchus and lung). ICD-O, 157 Malignant neoplasm of pancreas.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Unblinded interview using questionnaire sought information on complete job history with supplemental questionnaire for jobs of *a priori* interest (e.g., machinists, painters). Team of chemist and industrial hygienist assigned exposure using job title with a semiquantitative scale developed for 294 exposures, including TCE. For each exposure, a 3-level ranking was used for concentration (low or background, medium, high) and frequency (percent of working time: low, 1 to 5%; medium, >5 to 30%; and high, >30%).

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	82% of all cancer cases interviewed face-to-face by a trained interviewer, 10% telephone interview, and 8% mailed questionnaire. Cases interviews were conducted either at home or in the hospital; all population control interviews were conducted at home.
Blinded interviewers	Interviews were unblinded but exposure coding was carried out blinded as to case and control status.
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Yes, 20% of all cancer cases had proxy respondents.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	857 lung cancer cases (79.2% response), 117 pancreatic cancer cases (70.7% response); 533 population controls (72% response). Exposure prevalence: Any TCE exposure, 2% cancer cases (n = 21 lung cancer cases and 2 pancreatic cancer cases); substantial TCE exposure (exposure for $\geq$10 yrs and up to 5 yrs before disease onset), 1% lung cancer cases (n = 9), no pancreatic cancer cases assigned “substantial” TCE exposure.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Lung cancer—age, family income, cigarette smoking, ethnic origin, alcohol consumption, and respondent status. Pancreatic cancer —age, income, cigarette smoking, and respondent status.
Statistical methods	Mantel-Haenszel (Siemiatycki, 1991).
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3. Geographic-Based Studies

B.3.3.1. *Coyle et al. (2005)*

B.3.3.1.1. *Author's abstract.*

> Purpose. To investigate the role of environment in breast cancer development, we conducted an ecological study to examine the association of releases for selected industrial chemicals with breast cancer incidence in Texas.
> Methods. During 1995–2000, 54,487 invasive breast cancer cases were reported in Texas. We identified 12 toxicants released into the environment by industry that: (1) were positively associated with breast cancer in epidemiological studies, (2) were Environmental Protection Agency (EPA) Toxics Release Inventory (TRI) chemicals designated as carcinogens or had estrogenic effects associated with breast cancer risk, and (3) had releases consistently reported to EPA TRI for multiple Texas counties during 1988–2000. We performed univariate, and multivariate analyses adjusted for race and ethnicity to examine the association of releases for these toxicants during 1988–2000 with the average annual age-adjusted breast cancer rate at the county level.
> Results. Univariate analysis indicated that formaldehyde, methylene chloride, styrene, tetrachloroethylene, trichloroethylene, chromium, cobalt, copper, and nickel were positively associated with the breast cancer rate. Multivariate analyses indicated that styrene was positively associated with the breast cancer rate in women and men ($b = 0.219$, $p =0.004$), women ($b = 0.191$, $p=0.002$), and women ‡ 50 years old ($b = 0.187$, $p=0.002$).
> Conclusion. Styrene was the most important environmental toxicant positively associated with invasive breast cancer incidence in Texas, likely involving women and men of all ages. Styrene may be an important breast carcinogen due to its widespread use for food storage and preparation, and its release from building materials, tobacco smoke, and industry.

B.3.3.1.2. ***Study description and comment.*** Residential address in 254 Texas counties at time of cancer diagnosis was the exposure surrogate in this ecologic study of invasive breast cancer in over a 5-year period (1995−2000). Incident breast cancer cases in males and females were identified from Texas Cancer Registry. During the 5-year period, 54,487 cases were diagnosed, of which 53,910 were in females (99%). Median average annual age-adjusted breast cancer rates for women and men, women, women <50 years old, and women ≥50 years old and 12 hazardous air pollutants identified as exposures of interested were examined using nonparametric tests (Mann-Whitney U test) and linear regression analyses. The 12 hazardous air pollutants (HAPs) were: carbon tetrachloride, formaldehyde, methylene chloride, styrene, perchloroethylene, TCE, arsenic, cadmium, chromium, cobalt, copper, and nickel. On-site atmospheric release data on individual HAPs was identified from EPA's Toxics Release Inventory (TRI) for a 13-year period, 1998 to 2000 with an exposure surrogate as the annual total release in pounds/year for the 12 HAPs.

Coyle et al. (2005) compared average annual age-adjusted breast cancer rate for counties reporting a release to that rate for non-reporting counties using Mann-Whitney U test. Additionally, multiple linear regression analyses was used to determine the association of the average annual age-adjusted breast cancer rates with the 12 HAPs, adjusting for race and ethnicity when associated with the study's outcome variable.

While this study provides insight on cancer rates in studied population, TCE and other hazardous air pollutant exposures are poorly defined and the exposure surrogate unable to distinguish subjects more with higher exposure potential from those with low or minimal exposure potential. Some information may be provided through examination of inter-county release rates; however, no information is provided by Coyle et al. (2005). Furthermore, the ecologic design of the study does not address residential history or other information on an individual-subject level and is subject to bias from "ecologic fallacy" or improper inference about individual-level associations based on aggregate-level analysis. Overall, this study is not able to identify risk factors (etiologic exposures), has low sensitivity for examining TCE, and provides little weight in an overall weight of evidence evaluation of TCE and cancer.

Coyle YM, Hynan LS, Euhus DM, Minhajuddin ATM. 2005. An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. Breast Cancer Res Treat.92:107-114.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Hypothesis of this study was to evaluate breast risks in Texas counties and hazardous air pollutants (HAPs).
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases are incident breast cancers in males and females over a 5-yr period (1995−2000) in subjects residing in Texas and reported to the Texas Cancer Registry.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in Texas county as time of diagnosis is exposure surrogate. Annual release by county of 12 HAPs (carbon tetrachloride, formaldehyde, methylene chloride, styrene, perchloroethylene, TCE, arsenic, cadmium, chromium, cobalt, copper, and nickel) are obtained from EPA's TRI database.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	54,487 incident breast cancer cases in males and females.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and race/ethnicity.
Statistical methods	Mann-Whitney U test (nonparametric) to compared average annual age-adjusted breast cancer rate between counties reported HAP release to that for non-reporting counties. Linear logistic regression
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

EPA = Environmental Protection Agency. HAP = hazardous air pollutant. TRI = Toxic Release Inventory.

B.3.3.2. *Morgan and Cassady (2002)*

B.3.3.2.1. *Author's abstract.*

> In response to concerns about cancer stemming from drinking water contaminated with ammonium perchlorate and trichloroethylene, we assessed observed and expected numbers of new cancer cases for all sites combined and 16 cancer types in a California community (1988 to 1998). The numbers of observed cancer cases divided by expected numbers defined standardized incidence ratios (SIRs) and 99% confidence intervals (CI). No significant differences between observed and expected numbers were found for all cancers (SIR, 0.97; 99% CI, 0.93 to 1.02), thyroid cancer (SIR, 1.00; 99% CI, 0.63 to 1.47), or 11 other cancer types. Significantly fewer cases were observed than expected for cancer of the lung and bronchus (SIR, 0.71; 99% CI, 0.61 to 0.81) and the colon and rectum (SIR, 0.86; 0.74 to 0.99), whereas more cases were observed for uterine cancer (SIR, 1.35; 99% CI, 1.06 to 1.70) and skin melanoma (SIR, 1.42; 99% CI, 1.13 to 1.77). These findings did not identify a generalized cancer excess or thyroid cancer excess in this community.

B.3.3.2.2. ***Study description and comment.*** Residential address in 13 census tracts in Redlands (San Bernardino County, CA) at time of cancer diagnosis was the exposure surrogate in this ecologic study of cancer incidence over a 10-year period (1988−1998). Seventeen cancers in adults (all cancers, bladder, brain and other nervous system, breast [females only], cervix, colon and rectum, Hodgkin lymphoma, kidney and renal pelvis, leukemia [all], liver and bile duct, lung and bronchus, NHL, melanoma, ovary, prostate, thyroid and uterus) and 3 site-specific incident cancers in children under 15 years of age (leukemia [all], brain/CNS, and thyroid) were identified from the Desert Sierra Cancer Surveillance Program, a regional cancer registry reporting to the California Cancer Registry, with expected numbers of site-specific cancer using age-race annual site-specific cancer incidence rates between 1988 and 1992 to 1990 census-reported information on population size and demographics. The use of the Desert Sierra Cancer Surveillance Program rates which include the studied population would inflate the number of site-specific cancer expected; however, the potential magnitude of bias is likely minimal given the Redlands populations was estimated as 2% of the total population of the regional cancer registries ascertainment area (Morgan and Cassidy, 2002). This is a record-based study and information on personal habits and potential risk factors other than race, sex, and age are lacking for individual subjects.

Morgan and Cassidy (2002) identified TCE and perchlorate from drinking water as exposures of interest. Limited monitoring data from the 1,980 identified TCE concentrations in Redlands wells as between 0.09 and 97 ppb TCE and drinking water concentrations as below the maximum contaminant level (MCL; 5 ppb) since 1991. The paper lacks information if water monitoring represented wells in the 13-census tract study area. Furthermore, the paper does not include information on water treatment and distribution networks to provide an estimate of TCE concentration in finished tap water to individual homes. These authors noted their inability to identify higher or lower exposed subjects, as well, as minimally exposed subjects as a source of uncertainty. No data are presented on perchlorate concentrations in well or drinking water. The assumption of residence in 13 census tracts is insufficient as a surrogate of potential exposure to TCE and perchlorate in the absence of exposure modeling and data on water distribution patterns. Exposure misclassification bias is highly likely and of a nondifferential nature which would dampen observed associations.

While this study provides insight on cancer rates in studied population, TCE exposure is poorly defined and the exposure surrogate unable to distinguish subjects more with higher exposure potential from those with low or minimal exposure potential. Furthermore, the ecologic design of the study does not address residential history or other information on an individual-subject level and is subject to bias from "ecologic fallacy" or improper inference about individual-level associations based on aggregate-level analysis. Morgan and Cassidy (2002) furthermore discuss the relatively high education and income levels in the Redlands population compared with the average for the referent population may lead to lower tobacco use and higher than average access to health care, biases that would dampen risks for lung and other tobacco-related cancers, but may also increase risks for colon and cervical cancers. Overall, this study is not able to identify risk factors (etiologic exposures), has low sensitivity for examining TCE, and provides little weight in an overall weight of evidence evaluation of TCE and cancer.

Morgan JW, Cassady RE. 2002. Community cancer assessment in response to long-time exposure to perchlorate and trichloroethylene in drinking water. J Occup Environ Med 44:616−621.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Hypothesis of this study was to evaluate cancer risks in a California community, not to evaluate TCE and cancer explicitly.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases are incident cancers over a 10-yr period (1988−1989) in subjects residing in 13 Redlands (CA) census tracts at time of diagnosis. 17 site-specific cancers are identified in adults and 3 site-specific cancers in children less than 15 yrs old. Cancer cases identified from Desert Sierra Cancer Surveillance Program (DSCSP), a regional cancer registry. Annual age-race-site specific cancer rates from DSCSP for 1988 and 1992 and age-race-sex specific population estimates for 1990.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in a 13-census tract area of Redlands, CA is exposure surrogate. No data are presented on TCE or perchlorate concentrations in treated drinking water supplied to residents.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	

CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	3,098 incident cancers, the largest number from 536 breast cancer and fewest number from Hodgkin disease.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and race/ethnicity.
Statistical methods	SIR with indirect standardization of estimated expected numbers of site-specific cancers adjusted for population growth; 90% confidence intervals presented in tables.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

SIR = standardized incidence ratio.

B.3.3.3. *Cohn et al. (1994)*

B.3.3.3.1. *Author's abstract.*

> A study of drinking water contamination and leukemia and non-Hodgkin's lymphoma (NHL) incidence (1979-1987) was conducted in a 75-town study area. Comparing incidence in towns in the highest trichloroethylene (TCE) stratum (>5 microg/L) to towns without detectable TCE yielded an age-adjusted rate ratio (RR) for total leukemia among females of 1.43 (95% CI 1.07-1.90). For females under 20 years old, the RR for acute lymphocytic leukemia was 3.26 (95% CI 1.27-8.15). Elevated RRs were observed for chronic myelogenous leukemia among females and for chronic lymphocytic leukemia among males and females. NHL incidence among women was also associated with the highest TCE stratum (RR = 1.36; 95% CI 1.08-1.70). For diffuse large cell NHL and non-Burkitt's high-grade NHL among females, the RRs were 1.66 (95% CI 1.07-2.59) and 3.17 (95% CI 1.23-8.18), respectively, and 1.59 (95% CI 1.04-2.43) and 1.92 (95% CI 0.54-6.81), respectively, among males. Perchloroethylene (PCE) was associated with incidence of non-Burkitt's high-grade NHL among females, but collinearity with TCE made it difficult to assess relative influences. The results suggest a link between TCE/PCE and leukemia/NHL incidence. However, the conclusions are limited by potential misclassification of exposure due to lack of individual information on long-term residence, water consumption, and inhalation of volatilized compounds.

B.3.3.3.2. ***Study description and comment.*** This expanded study of a previous analysis of TCE and perchloroethylene in drinking water in a 27-town study area (Fagliano et al., 1990) examined leukemia and NHL incidence from 1979 to 1987 in residents and TCE and other VOCs in drinking water delivered to 75 municipalities. Exposure estimates were developed from data generated by a mandatory monitoring program for four trihalomethane chemicals and 14 other volatile organic chemicals in 1984−1985 for public water supplies and from historical monitoring data conducted in 1978−1984 by the New Jersey Department of Environmental Protection and Energy and the New Jersey Department of Health, which was the mean of monthly averages for this period. The average and maximum concentration of TCE and other chemicals were estimated by considering together, for the period prior to 1985, details of the distribution system size, well or surface water use, patterns of water purchases among systems, and significant changes in water supply, and for years after 1985, samples of finished water from the plant and samples taken from the distribution system under the assumption of homogeneous mixing. The number of distribution system samples for each supply varied from 2 to 50. Additionally, a dilution factor assuming complete mixing was used to adjust for water purchased from another source. A single summary average and maximum concentration for each contaminate for a municipality was assigned to all cases residing in that municipality at the time of cancer diagnosis. Concentrations of TCE and perchloroethylene were highly correlated ($r = 0.63$). A ranking of municipalities was the same when using average or maximum concentration and the maximum concentration of TCE or perchloroethylene used in statistical analyses was grouped into three strata: <0.1 ppb (referent group), 0.1−5 ppb, >5−20 ppb, and >20 ppb.

Incident cases of NHL and forms of leukemia reported to the New Jersey State Cancer Registry were identified from 1979 and 1987. Incidence rate ratios were estimated using Poisson regression models fitted to age- and sex-specific numbers of cases by exposure strata and the stratum-specific population. Statistical treatment considered exposure to other drinking water contaminants, atmospheric emissions of hazardous air pollutants as reported to U.S. EPA's Toxics Release Inventory (TRI) by municipality and two socioeconomic variables measured as municipal—average annual household income and percentage of high school graduates. None of the water trihalomethane or volatile organic contaminants other than perchloroethylene was shown to be associated with childhood leukemia or adult lymphomas. Furthermore, neither average income, education, nor TRI release data were associated with NHL or leukemia except in one exception, TRI release was shown to modify the effects of TCE and high-grade non-Burkett's lymphoma in females.

This ecological study is subject to known biases and confounding as introduced through its study design (NRC, 1997). Exposure estimates are crude (averages), do not consider individual differences in drinking water patterns, and assigns group exposure levels to all subjects without consideration of residential history. Potential for misclassification bias is likely great in this study as is the potential for bias. This study does attempt to examine three possible confounding exposures, although these are crudely defined, and some potential for residual confounding is possible given the study's use of aggregated data.

Cohn P, Klotz J, Bove F, Berkowitz M, Fagliano J. 1994. Drinking water contamination and the incidence of leukemia and non-Hodgkin's lymphoma. Environ Health Perspect 102:556−561.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This study was designed to further examine drinking water contaminates and lymphoma; a previous study of TCE and perchloroethylene in drinking water found a statistically significant association with leukemia among females residing in a 27-town study area (Fagliano et al., 1990).
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Incident cases of various forms of leukemia (all leukemia, acute lymphocytic, chronic lymphocytic, acute myelogenous, chronic myelogenous, other specified and unspecified leukemia) and NHL (total, low-grade, intermediate-grade [total and diffuse large cell a B-cell lymphoma], high-grade including non-Burkett's lymphoma) from 1979−1987 are identified from New Jersey State Cancer Registry. Subjects grouped in lowest exposure category are referents.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Average and maximum concentration of TCE and other chemicals were estimated by considering together, for the period prior to 1985, details of the distribution system size, well or surface water use, patterns of water purchases among systems, and significant changes in water supply, and for years after 1985, samples of finished water from the plant and samples taken from the distribution system under the assumption of homogeneous mixing. No difference in municipality ranking by average or maximum concentration. Three grouped categories of maximum concentration in statistical analysis are <0.1 ppb (referent), 0.1−5 ppb, >5 ppb (U.S. EPA Maximum Contaminant Level for TCE and perchloroethylene).
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	1,190 leukemia cases (663 males, 527 females), 119 cases assigned >5.0 ppb TCE. 1,658 NHL cases (841 males, 817 females), 165 cases assigned >5.0 ppb TCE.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex.
Statistical methods	Poisson regression fitted to the age-and sex-specific count of cases in towns grouped by exposure strata and weighted by the logarithm of the strata-specific population.

Exposure-response analysis presented in published paper	Yes.
Documentation of results	Yes.

B.3.3.4. *Vartiainen et al. (1993)*

B.3.3.4.1. *Author's abstract.*

> Concentrations up to 212 μg/l of trichloroethene (TCE) and 180 μg/l of tetrachloroethene (TeCE) were found in the drinking water from two villages in Finland. To evaluate a possible exposure, urine sample fro m95 and 21 inhabitants in these villages and from two control groups of 45 and 15 volunteers were collected. Dichloroacetic acid (DCA) and trichloroacetic acid (TCA), the metabolites of TCE and TeCE, were also analyzed. The individuals using contaminated water in one of the villages excreted TCE an average 19 μg/d (<1 – 110 μg/d) and in the other 7.9 μg/d (<1 – 50 μg/d), while the controls excreted an average 2.0 μg/d (<1 – 6.4 μg/d) or 4.0 μg/d (<1 – 13 μg/d). No increased incidence rates were found in the municipalities in question for total cancer, liver cancer, non-Hodgkin's lymphomas, Hodgkin's disease, multiple myeloma, or leukemia.

B.3.3.4.2. ***Study description and comment.*** This published study of two separate analyses, (1) urinary biomonitoring of 106 subjects from two Finish municipalities, Hausjärvi and Hattula, and, (2) calculation of total cancer and site-specific cancer incidence between 1953 and 1991 in Hausjärvi and Hattula residents. Limited exposure monitoring data are presented in the paper. TCE concentrations in drinking water from Oitti are lacking other than noting TCE and perchloroethylene were 100−200 μg/L in 1992. TCE concentrations in drinking water from Hattula were below 10 μg/L in December 1991; however, samples (number unknown) taken 6 months later contained 212 μg/L and 66 μg/L TCE. These two municipalities discontinued use of these sources for drinking water in August 1992.

Cancer incidence for 6 sites (all cancers, liver cancer, NHL, Hodgkin's lymphoma, multiple myeloma, and leukemia) between 1953−1991 in Hausjärvi and Hattula residents was obtained from the Finnish Cancer Registry. A total of 1,934 cancers were observed during the study period. Standardized incidence ratios for each municipality were calculated using site-specific cancer incidence rates from the Finnish population for the entire time period and for 3 shorter periods, 1953−1971, 1972−1981, and 1982−1991. The paper does not identity the source for or size of Hausjärvi and Hattula population estimates and if temporal changes in population estimates were considered in the statistical analysis. This study using record systems did not include information obtained directly from subjects and lacks information on personal and lifestyle factors that may introduce bias or confounding.

This study provides little information in an overall weight-of-evidence analysis on cancer risks and TCE exposure. A major limitation is its lack of exposure assessment to TCE and perchloroethylene. While this study provides some information on cancer incidence in the two towns over a 40-year period, this study is not able to identify potential risk factors and exposures.

Vartiainen T, Pukkala E, Rienoja T, Strandman T, Kaksonen K. 1993. Population exposure to tri- and tetrachloroethene and cancer risk: two cases of drinking water pollution. Chemosphere 27:1171−1181.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Study aim was (1) to determine if residents of two villages in Finland had exposure to TCE and perchloroethylene as indicated from urinary biomonitoring, (2) identify biomarker for low-level exposure, and (3) to determine cancer incidence in Hausjärvi and Hattula, two municipalities in Finland. This study could not identify potential risk factors.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cancer incidence cases identified from Finnish Cancer Registry. Site-specific cancer rates for the Finnish population was used a referent.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in two municipalities is the exposure surrogate in this ecologic study. The paper lacks exposure assessment to TCE and perchloroethylene in drinking water in Hausjärvi and Hattula.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	3,846 cancer cases; 1,942 from Hausjärvi and 1,904 from Hattula.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex.
Statistical methods	SIR with cancer incidence rates in Finnish population as referent.
Exposure-response analysis presented in published paper	No.
Documentation of results	Cancer incidence analysis is not well documented.

SIR = standardized incidence ratio.

B.3.3.5. *Mallin (1990)*

B.3.3.5.1. *Author's abstract.*

> Cancer maps from 1950 through 1979 revealed areas of high mortality from bladder cancer for both males and females in several northwestern Illinois counties. In order to further explore this excess, a bladder cancer incidence study was conducted in the eight counties comprising this region. Eligible cases were those first diagnosed with bladder cancer between 1978 and 1985. Age adjusted standardized incidence ratios were calculated for each county and for 97 zip codes within these counties. County results revealed no excesses. Zip code results indicated elevated risks in a few areas, but only two zip codes had significantly elevated results. One of these zip codes had a significant excess in males (standardized incidence ratio = 1.5) and females (standardized incidence ratio = 1.9). This excess was primarily confined to one town in this zip code, in which standardized incidence ratios were significantly elevated in males (1.7) and females (2.6). Further investigation revealed that one of four public drinking water wells in this town had been closed due to contamination; two wells were within a half mile (0.8 km) of a landfill site that had ceased operating in 1972. Tests of these two wells revealed traces of trichloroethylene, tetrachloroethylene, and other solvents. Further investigation of this cluster is discussed.

B.3.3.5.2. ***Study description and comment.*** This ecologic study of bladder cancer incidence and mortality among white residents in nine Illinois counties between 1978−1985 was carried out to further investigate a previous finding of elevated bladder cancer mortality rates in some counties. The study lacks exposure assessment to subjects and potential sources of exposure was examined in a *post hoc* manner in one case only, for a community with an observed elevated bladder cancer incidence. The limited exposure examination focused on groundwater contamination and proximity of Superfund sites to the community, lacked assignment of exposure surrogates to individual study subjects, and findings are difficult to interpret given the lack of exposure assessment for the other eight counties.

Histologically-confirmed incident bladder cancer cases were identified from hospital records in eight of the nine counties. Since the 9-county area bordered on neighboring states of Wisconsin and Iowa, incident bladder cancer cases were also ascertained from the Wisconsin Cancer Reporting System and Iowa's State Health Registry. No information is provided in the paper on completeness of ascertainment of bladder cancer cases among residents or on the source for identifying bladder cancer deaths. Expected numbers of incident cancers calculated using age-specific rates for white males and females from the SEER program (incidence) or the United States population [mortality], and the census data on population estimates for the nine-county area. Statistical analyses adopt indirect standardization methods to calculate SMR and standardized incidence ratios (SIRs) for a community and SIRs for individual postal zip codes. The use of records and absence of information collected from subject personal interviews precluded examination of possible confounders other than age and race.

This ecological study is subject to known biases and confounding as introduced through its study design (NRC, 1997). Ecological studies like this study are subject to bias known as "ecological fallacy" since variables of exposure and outcome measured on an aggregate level may not represent association at the individual level. Consideration of this bias is important for diseases with more than one risk factor, such as the site-specific cancers evaluated in this assessment. Lack of information on smoking is another uncertainty. While this study provides insight on bladder cancer rates in the studied communities, it does not provide any evidence on cancer and TCE exposure. For this reason, this study provides little weight in an overall weight-of-evidence analysis.

Mallin K. 1990. Investigation of a bladder cancer cluster in Northwestern Illinois. Amer J Epidemiol 132:S96−S106.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	The hypothesis of study was to "further exposure a previous finding of bladder cancer excess in several northwestern Illinois counties." (from abstract).
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Incident cancer cases diagnosed between 1978−1985 were identified in residents in 9 northwestern Illinois counties from the Illinois Cancer Registry, the Wisconsin Cancer Reporting System or the Iowa State Health Registry. Source for deaths in subjects residing at the time of death in the 9 counties was not identified in the published paper. Expected number of bladder cancer derived using (1) SEER age-race-sex specific incidence rates and (2) age-race-sex specific mortality rates of the U.S. population for 1978−1981 and for 1982−1985 and census estimates of population for each county or postal zip code area.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence and mortality.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This is a health survey and lacks exposure assessment to communities and to individual subjects. Monitoring of volatile organic chemicals including trichloroethylene in two municipal drinking water wells for 1982−1988 in a community with elevated bladder cancer rates was identified in paper; TCE concentrations were less than 15 ppb. It is not know whether monitoring data are representative of exposure to study subjects.

CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	712 bladder cancer incident cases and 222 bladder cancer deaths among white males and female residents in nine northwestern Illinois counties.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex .
Statistical methods	SIR with cancer incidence rates from Surveillance, Epidemiology and End Results program and mortality rates of U.S. population as referents.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.6. *Issacson et al. (1985)*

B.3.3.6.1. *Author's abstract.*

> With data from the Iowa Cancer Registry, age-adjusted sex-specific cancer incidence rates for the years 1969-1981 were determined for towns with a population of 1,000–10,000 and a public water supply from a single stable ground source. These rates were related to levels of volatile organic compounds and metals found in the finished drinking water of these towns in the spring of 1979. Results showed association between 1,2 dichloroethane and cancers of the colon and rectum and between nickel and cancers of the bladder and lung. The effects were most clearly seen in males. These associations were independent of other water quality and treatment variables and were not explained by occupational or other sociodemographic features including smoking. Because of the low levels of the metals and organics, the authors suggest that they are not causal factors, but rather indicators of possible anthropogenic contamination of other types. The data suggest that water quality variables other than chlorination and trihalomethanes deserve further consideration as to their role in the development of human cancer.

B.3.3.6.2. ***Study description and comment.*** This ecologic study of cancer incidence at six sites [bladder, breast, colon, lung, prostate, rectum] and chlorinated drinking water uses monitoring data from finished public drinking water supplies to infer exposure to residents of Iowa towns of 1,000−10,000 population sizes. Towns were included if they received water from a single major source (surface water, wells of <150 feet depth, or wells ≥50 feet depth) prior to 1965. Water monitoring for VOCs, trace elements and heavy metals was carried in Spring, 1979, as part of a larger nation-wide collaborative study of bladder cancer and artificial sweeteners (Hoover and Strasser, 1980), and samples analyzed using proton-induced x-ray emission for trihalomethanes, TCE, perchloroethylene, 1,2-dichloroethane, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethylene, and 43 inorganic elements. 1,1,1-trichloroethane was the most frequently detected VOC in both surface and groundwater; TCE, perchloroethylene, and 1,2-dichloroethane were more frequently detected in shallow wells than in deep (>150 feet) wells.

Cancer incidence was obtained for the period 1969 and 1981 with age-adjusted site-specific cancer incidence rates for males and females calculated separately for four VOCs (1,2-dichloroethane, TCE, perchloroethylene, and 1,1,1-trichloroethane) in finished groundwater supplies using the direct standardization method. Using the address at the time of diagnosis, each cancer patient was classified into one of two groups: (1) residing within the city limits and, thus, drinking the municipality's water, or (2) residing outside the city limits and consuming water from a private source. Age-adjusted incidence rates are reported by group study town into two TCE water concentrations categories of <0.15 μg/L and ≥0.15 μg/L.

This ecological study on drinking water exposure and cancer provides little information in a weight-of-evidence analysis of TCE and cancer. Exposure estimates are crude (averages), do not consider individual differences in drinking water patterns or other sources of exposure, and assigns group exposure levels to all subjects. Potential for misclassification bias is likely great in this study, likely of a nondifferential nature, and dampen observations.

Isacson P, Bean JA, Splinter R, Olson DB, Kohler J. 1985. Drinking water and cancer incidence in Iowa. III. Association of cancer with indices of contamination. Amer J Epidemiol 121:856−869.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This ecological study was designed to examine consistency with the hypothesis of an association between cancer and chlorinated water through examination of other water contaminants besides water chlorination by-products and trihalomethanes.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Subjects are incident cases of cancer of the bladder, breast, prostate, lung rectum, and stomach reported to the Iowa Cancer Registry between 1969 and 1981 and, who resided in towns with a 1970 population of 1,000−10,000 and a public drinking water supply coming solely from a single major source (wells) prior to 1965. Age-adjusted site-specific incidence rates are calculated using the direct method and the 1970 Iowa population.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Not identified in paper.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	As part of another epidemiologic study on water chlorination and bladder cancer, finished drinking water samples from treatment plant were collected in Iowa municipalities with populations of 1,000 or larger in Spring 1979 and analyzed using proton induced x-ray emission for 4 trihalomethanes (chloroform, chlorodibromomethane, bromoform, dibromochloromethane), 7 VOCs (TCE, perchloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and cis- and trans-1,2-dichloroethylene) and 43 inorganic elements, including metals. The predominant contaminant was 1,1,1-trichloroethane; detectable levels of TCE were found in approximately 20% of sampled municipalities. Study towns were ranked into two categories of TCE in finished water, <0.15 µg/L and ≥0.15 µg/L in the statistical analysis.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	11,091 cancer cases of which ~20% of cases resided in municipality with finished water TCE concentration of ≥0.15 µg/L. Bladder, 852 cases Breast (female), 1,866 cases Colon, 2,032 cases Lung 1,828 cases Prostate, 1,823 cases Rectum, 824 cases

CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex.
Statistical methods	Age-adjusted site-specific mortality rates calculated using direct standardization method and 1970 Iowa population.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.7. *Studies in the Endicott Area of New York*

A series of health statistics reviews and exposure studies have been conducted in an area with a history of VOCs, including trichloroethylene, detected in municipal wells used to supply drinking water to residents of Endicott, Broome County, NY. These studies were carried out by staff the New York State Department of Health (NYS DOH) with support from the ATSDR. Early health surveys examined cancer incidence among Broome County residents between 1976−1980 or 1981−1990, with focused analyses of cancer incidence among residents of Endicott Village and other nearby towns, childhood leukemia in the Town of Union and possible etiologic factors, and adult leukemia deaths and employment in the shoe and boot manufacturing industry (Forand, 2004; NYS DOH, 2008). Two recent studies focused on cancer incidence or birth outcomes among Village of Endicott residents living in a geographically defined area with VOC exposure potential as documented from indoor and soil vapor monitoring (ATSDR, 2006a, b, 2008).

The Village of Endicott is a mixed residential, commercial, and industrial community with a rich industrial heritage and a number of VOCs were used at industrial locations in and around Endicott, as well as, having been disposed at area landfills (ATSDR, 2006b). Three wells provide drinking water to the Village of Endicott: Ranney, which supplied most of the water used by the Endicott Municipal Water Works since it was first placed in service in 1950; and, South Street, where two wells resided. The Endicott Municipal Water Supply operates on a grid-water system, neighborhoods closest to the wells are usually supplied at a greater rate from nearby wells as compared to wells farther away (ATSDR, 2006b).

Routine monitoring of the Ranney well in the early 1980s detected VOCs at levels above New York State drinking water guidelines (ATSDR, 2006b). A groundwater contaminate plume northwest of the Ranney Well was found in a lower aquifer from which the municipal drinking supply is drawn. Several sources were initially recognized as contributing to contamination of the wellfield with a supplemental remedial investigation concluding that the Endicott Village Landfill was the source of the VOCs in the Endicott Wellfield water supply (ATSDR, 2006a). Groundwater water samples collected from monitoring wells installed during previous investigations, wells install as part of the supplemental remedial investigation, the Purge well, and the Ranney well contained many VOCs. Remediation efforts starting in the 1980s have reduced contamination in this well to current MCLs. Water monitoring of the South Street wells (wells 5 and 28) has been carried out for VOCs since 1980 and 1981, respectively (ATSDR, 2006b). Detection limits for VOCs from the South Street wells varied from 0.5−1.0 μg/L; 1,1-dichloroethane had the highest detection frequency, in 44% of all samples and TCE was detected in 3 of 116 samples obtained between 1980 and 2004 (ATSDR, 2006b).

An upper aquifer with a contaminant plume containing VOCs was also identified and sampling data indicated there were multiple sources of vapor contamination including a former IBM facility located in the Village (U.S. EPA, 2005; NYSDEC, 2007). This groundwater contaminant plume flows directly beneath the center of the Village of Endicott and serves as a source of soil vapor contamination. Findings of a 2002 investigation indicated vapor migration had resulted in detectable levels of contaminants in indoor air structures, including locations in the Village of Endicott and Town of Union. Of soil gas and indoor air monitoring at more than 300 properties in an area south of the IBM Endicott facility, TCE was the most commonly found contaminant in indoor air, at levels ranging from 0.18 to 140 (NYSDEC, 2007). This area is identified as the Eastern study area in the health statistics review of ATSDR (2006a, 2008). Other contaminants besides TCE detected in soil gas and indoor air less frequently and at lower levels included tetrachloroethylene, cis-1,2-dichloroethene, 1,1,1-trichloroethane, 1,1-dichloroethylene, 1,1-dichloroethane, and Freon 113. Vapor-intrusion contamination was also identified in a neighborhood adjacent to the Eastern area, call the Western study in the health statistic review, and perchloroethylene and its degradation by-products were detected by vapor monitoring. Perchloroethylene levels generally ranged from 0.1 to 3.5 $\mu g/m^3$ of air (ATSDR, 2006a).

B.3.3.7.1. ***Agency for Toxic Substances and Disease Registry (ATSDR, 2006a, 2008).***

B.3.3.7.1.1. ***Agency for Toxic Substances and Disease Registry (ATSDR, 2006a) executive summary***.

> ***Background*** The New York State Department of Health (NYS DOH) conducted this Health Statistics Review because of concerns about health issues associated with environmental contamination in the Endicott area. Residents in the Endicott area may have been exposed to volatile organic compounds (VOCs) through a pathway known as soil vapor intrusion. Groundwater in the Endicott area is contaminated with VOCs as a result of leaks and spills associated with local industry and commercial businesses. In some areas of Endicott, VOC contamination from the groundwater has contaminated the adjacent soil vapor which has migrated through the soil into structures through cracks in building foundations (soil vapor intrusion). Trichloroethene (TCE), tetrachloroethene (PCE) and several other VOCs have been found in the soil vapor and in the indoor air of some structures.
>
> ***Conclusions*** This health statistics review was conducted because of concerns that exposure to VOCs through vapor intrusion may lead to adverse health effects. Although this type of study cannot prove whether there is a causal relationship between VOC exposure in the study area and the increased risk of several health outcomes observed, it does serve as a first step in providing guidance for further

health studies and interventions. The elevated rates of several cancers and birth outcomes observed will be evaluated further to try to identify additional risk factors which may have contributed to these adverse health outcomes.

Limitations in the current study included limited information about the levels of VOCs in individual homes, the duration of the exposure, the amount of time residents spent in the home each day and the multiple exposures and exposure pathways that likely existed among long term residents of the Endicott area. In addition, personal information such as medical history; dietary and lifestyle choices such as smoking and drinking; and occupational exposures to chemicals were not examined. Future evaluations of cancer and birth defects and VOC exposures in the area should take these factors into account. The small population size of the study area also limited the ability to detect meaningful elevations or deficits in disease rates, especially for certain rare cancers and birth outcomes.

This study represents the first step in a step-wise approach to addressing health outcome concerns related to environmental contamination in Endicott, NY. Follow-up will consist of further reviewing of the cancer and birth outcome data already collected. Additional efforts will include reviewing individual case records of kidney and testicular cancers, heart defects, Down syndrome and term low birth weight births. In addition, we will review spontaneous fetal deaths among residents of the area. The information gained, along with the results of this Health Statistics Review, will be used to assess if a follow up epidemiologic study is feasible. Any follow-up study should be capable of accomplishing one of two goals: either to advance the scientific knowledge about the relationship between VOC exposure and health outcomes; or as part of a response plan to address community concerns. While not mutually exclusive, the distinction between these goals must be considered when developing a follow-up approach. Any plans for additional study will need to address other risk factors for these health outcomes such as smoking, occupation and additional information on environmental exposures. As in the past, NYS DOH will solicit input from the community.

B.3.3.7.1.2. ***Agency for Toxic Substances and Disease Registry (ATSDR, 2008) executive summary***.

This follow-up investigation was conducted to address concerns and to provide more information related to elevated cancers and adverse birth outcomes identified in the initial health statistics review entitled “Health Statistics Review: Cancer and Birth Outcome Analysis, Endicott Area, Town of Union, Broome County, New York” (ATSDR; 2006a).

The initial health statistics review was carried out to address concerns about health issues among residents in the Endicott area who may have been exposed to volatile organic compounds (VOCs) through a pathway known as soil vapor intrusion. The initial health statistics review reported a significantly elevated incidence of kidney and testicular cancer among residents in the Endicott area. In addition, elevated rates of heart defects and low birth weight births were observed. The number of term low birth weight births, a subset of low birth weight births, and the number of small for gestational age (SGA) births were also significantly higher than expected.

The purpose of this follow-up investigation was to gather more information and conduct a qualitative examination of medical and other records of individuals identified with adverse birth outcomes and cancers found to be significantly elevated. Quantitative analyses were also carried out for two additional birth outcomes, conotruncal heart defects (specific defects of the heart's outflow region), and spontaneous fetal deaths (stillbirths), and for cancer incidence accounting for race.

Cancer Incidence Adjusting for Race: Because a higher percentage of the population in the study area was white compared to the comparison population, we examined the incidence of cancer among whites in the study area compared to the incidence in the white population of New York State, excluding New York City. Cancer incidence among whites was evaluated for the years 1980-2001. Results: Limiting the analysis of cancer to only white individuals had little effect on overall cancer rates or standardized incidence ratios compared to those of the entire study area population analyzed previously. The only difference was the lung cancer which had been borderline non-significantly elevated was not borderline significantly elevated.

Cancer Case Record Review: We reviewed medical and other records of individuals with kidney and testicular cancers to try to determine smoking, occupational and residential histories. A number of preexisting data sources were used including: hospital medical records; cancer registry records; death certificates; newspaper obituaries; Motor Vehicle records; and city and telephone directories. Results: The case record review did not reveal any unusual patterns in terms of age, gender, year of diagnosis, cell type, or mortality rate among individuals with kidney or testicular cancer. There was some evidence of an increased prevalence of smoking among those with kidney cancer and some indication that several individuals diagnosed with testicular and kidney cancer may have been recent arrivals to the study area.

Conclusions/Recommendations: The purpose of the additional analyses reported in the draft for public comment follow-up report was to provide information on certain cancers and reproductive outcomes which were elevated in the initial health statistics review. Although these additional analyses could not determine whether there was a causal relationship between VOC exposures in the study area and the increased risk of several health outcomes that were observed, they did provide more information to help guide additional follow-up. The March 2007 public comment report provided a list of follow-up options for consideration and stated, "Although an analytical (case-control) epidemiologic study of cancer or birth defects within this community is not recommended at this time, we describe several follow up options for discussion with the Endicott community. A case-control study would be the preferable method for progressing with this type of investigation, but the potentially exposed population in the Endicott area is too small for conducting a study that would be likely to be able to draw strong conclusions about potential health risks.

Alternative follow-up options were discussed at meetings with Endicott stakeholders and were the subject of responses to comments on the draft report. From these discussions and written responses, NYS DOH has noted community interest in two possible options for future activities: a health statistics review based on historic outdoor air emissions modeling, and a multi-site epidemiologic study examining cancer outcomes in communities across the state with VOC exposures similar to Endicott. NYS DOH has considered these comments and examined whether these options would be able to accomplish one of two goals: either to advance the scientific knowledge about the relationship between VOC exposure and health outcomes or to be part of a response plan to address community concerns.

An additional health statistics review using historic outdoor air emission modeling results to identify and study a larger population of residents potentially exposed to TCE is not likely to meet either of these goals at this time. Because of the limitations of the health statistics review for drawing conclusions about cause and effect, conducting an additional health statistics review is not likely to increase our understanding of whether exposures in the Endicott area are linked to health outcomes. Limitations with the available historic outdoor air data also would make it difficult to accurately define the appropriate boundaries for the exposure area. ATSDR historic outdoor air emissions modeling activity was unable to model TCE due to a lack of available records.

A multi-site epidemiologic study of health outcomes in communities across the state with VOC exposures similar to Endicott offers some promise of meeting the goal of advancing the scientific knowledge about the relationship between VOC exposures and health outcomes. The community has indicated its preference that such a study focus on cancer outcomes. Given the complex issues involved in conducting such a study (e.g., tracking down cases or their next of kin after many years, participants' difficulty in accurately remembering possible risk factors from many years ago, and the long time period between exposure to a carcinogen and the onset of cancer), we do not consider a multisite case-control study of cancer as the best option at this time. An occupational cancer study is a better option than a community-based study because it can better incorporate information about past workplace exposures and could use corporate records to assist in finding individual employees many years after exposure.

Heart defects have been associated with TCE exposure in other studies. Given the shorter latency period, and thus the shorter time period in which other risk factors could come into play, a multi-site study of heart defects has some merit as a possible option. Currently, NYS DEC and NYS DOH are investigating many communities around New York State which could have VOC exposure patterns similar to Endicott, and thus could be included in such a multi-site epidemiologic study. However, in most of these communities exposure information sufficient to identify a study population is not yet available. NYS DOH will continue to evaluate these areas as additional exposure information becomes available, with the goal of identifying other communities for possible inclusion in a multi-site epidemiologic study of heart defects.

NYS DOH will continue to keep the Endicott community and stakeholders informed about additional information regarding other communities with exposures similar to those that occurred in the Endicott area. NYS DOH staff will be available as needed to keep interested Endicott area residents up-to-date on the feasibility of conducting a multi-site study that includes the Endicott area.

B.3.3.7.1.3. ***Study description and comment***. Health statistics review conducted by NYS DOH because of concerns about possible exposures to VOCs in Endicott area groundwater and vapor intrusion into residences examined cancer incidence between 1980 and 2001 and birth outcomes among residents living in a study area defined by soil vapor sampling and exposure modeling. The reviews were supported by ATSDR and conclusions presented in final reports (ATSDR, 2006a, 2008) have received external comment, but the studies have not been published in the open peer-reviewed literature. Testing of soil gas and indoor air of more than 300 properties, including 176 residences [location not identified] for VOCs detected TCE levels ranging from 0.18−140 $\mu g/m^3$; other VOCs less commonly detected included perchloroethylene, 1,1-dichloroethane, 1,1-dichloroethylene, 1,2-dichloroethylene, vinyl chloride, 1,1,1-trichloroethane, methylene chloride, and Freon 113. A model was developed to predict VOC presence in soil vapor based on measured results (Sanborn Head and Associates, 2003). Subsequent sampling and data collection verified this model. Initial study area boundaries were determined based on the extent of the probable soil vapor contamination greater than 10 $\mu g/m^3$ of VOCs as defined by the model. Contour lines of modeled VOC soil vapor contamination levels, known as isopleths, were mapped using a geographic information system. This study area is referred to as the Eastern study area in ATSDR (2006a, 2008). Additional sampling west of the initial study area identified further contamination with the contaminant in this area primarily identified as perchloroethylene at levels ranging from 0.1−3.5 $\mu g/m^3$ in an area referred to as the Western study area (ATSDR, 2006a, 2008). The source of perchloroethylene contamination was not known. A digital map of the 2000 Census block boundaries was overlaid on these areas of contamination. The study areas were then composed of a series of blocks combined to conform as closely to the areas of soil vapor contamination as possible.

Incident cancer cases for 18 sites, including cancer in children 19 years or younger, between 1980 and 2001 and obtained from the New York State Cancer Registry and addresses were geocoded to identify cases residing in the study area. The observed numbers of site-specific cancers were compared to that expected calculated using age-sex-year specific cancer incidence rates for New York State exclusive of New York City and population estimates 1980, 1990 and 2000 Censuses. Expected numbers of site-specific cancer did not include adjustment for race in ATSDR (2006a); however, race was examined in the 2008 follow-up study which compared cancer incidence among the white residents in the study area to that of whites in New York State (ATSDR, 2008). Over the 22-year period, a total of 347 incident cancers were observed among residents in the study area, 339 of these were in white residents. Less than 6 cases of cancers in children 19 years of age or younger were identified and ATSDR (2006a) did not present a SIR for this grouping, similar to their treatment of other site-specific cancers with less than six observed cases.

The follow-up analysis by ATSDR (2008) reviewed medical records of kidney and testicular cancer cases for smoking, occupational and residential histories, and restricted the statistical analysis to white residents, given the few numbers of observed cancers in the small population of nonwhite residents. Limiting the analysis to only white individuals in the study area had little effect on overall cancer rates or SIR estimates (ATSDR, 2006a). As observed in ATSDR (2006a), statistically significant excess risks were observed for kidney cancer in both sexes and testicular cancer in males. In addition, lung cancer estimate risks in males and in males and females were of the same magnitude in both analyses, but confidence intervals excluded a risk of 1.0 in the ATSDR (2008) analyses which adjusted for race. Review of medical records for the 15 kidney cancer and six testicular cancer cases provided limited information about personal exposures and potential risk factors because of incomplete reporting in records. The record review did not reveal any unusually patterns in either kidney cancer or testicular cancer in terms of age, year of diagnosis, anatomical site, cell type, or mortality rate. Occupational history suggested possible workplace chemical exposure for roughly half of the 13 kidney cancer cases and none of the testicular cancer cases whose medical records included occupational history. For smoking, half of the 9 kidney cancer cases and some (number not identified) of the 3 testicular cancer cases with such information in medical records were current or former smokers; smoking habits were not reported for the other cases. Last, examination of city and phone directories revealed while half the kidney cancer cases as long term Endicott residents, several cases of testicular cancer were among residents who recently moved into the Endicott area.

These health surveys are descriptive; they provide evidence of cancer rates in a geographical area with some documented exposures to several VOCs including trichloroethylene but are unable to identify possible etiologic factors for the observed elevations in kidney, testicular, or lung cancers. The largest deficiency is the lack of exposure assessment, notably historical exposure, to individual subjects. Review of city and phone directories suggests some kidney and testicular cancer cases were among recently-arrived residents, a finding inconsistent with a cancer latent period; however, of greater importance is the finding of cancers among subjects with long residential history. On the other hand, the population in the study areas has declined over the past 20 years (ATSDR, 2006a) and residents who may have moved from the study area were not included, introducing potential bias if cancer risks differed in these individuals. The medical history review suggests several risk factors including smoking and occupational exposure as important to kidney and testicular cancer observations. Lacking information for all subjects, there is uncertainty regarding the additive effect of other potential risk factors such as smoking to residential exposures. For this reason, while excesses in several incident cancers are observed in these reports, potential etiological risk factors are ill-defined, and the weight these studies contribute in the overall weight-of-evidence analysis is limited.

ATSDR (Agency for Toxic Substances and Disease Registry). 2006a. Health Consultation. Cancer and Birth Outcome Analysis, Endicott Area, Town of Union, Broome County, New York. Health Statistics Review. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. May 26, 2006.

ATSDR (Agency for Toxic Substances and Disease Registry). 2008. Health Consultation. Cancer and Birth Outcome Analysis, Endicott Area, Town of Union, Broome County, New York. Health Statistics Review Follow-Up. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. May 15, 2008.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	This health statistics review examined incidence for 18 types of cancer in residents living in the Village of Endicott at the time of diagnosis. This study was not designed to identify possible etiologic factors.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Subjects are incident cases of cancer of the 18 types of cancers including childhood cancer (all cancers in children ≤19 yrs of age) reported to the New York Cancer Registry between 1980 and 2001 among residents in two areas of the Village of Endicott, NY. The expected number of cancer cases for the period was calculated using cancer incidence rates for New York State exclusion of New York City and population estimates from 1980, 1990, and 2000 Censuses.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD 9th Revision.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	This geographic-based study does not develop quantitative estimates of exposure, rather study boundaries are defined using soil gas and indoor air monitoring data and computer modeling. Testing of soil gas and indoor air of more than 300 properties, including 176 residences (location not identified) in the Eastern study area for VOCs detected TCE levels ranging from 0.18−140 μg/m^3; other VOCs less commonly detected included perchloroethylene, 1,1-dichloroethane, 1,1-dichloroethylene, 1,2-dichloroethylene, vinyl chloride, 1,1,1-trichloroethane, methylene chloride, and Freon 113. A model was developed to predict VOC presence in soil vapor based on measured results (Sanborn Head and Associates, 2003). Subsequent sampling and data collection verified this model. Initial study area boundaries were determined based on the extent of the probable soil vapor contamination greater than 10 μg/m^3 of VOCs as defined by the model. Additional sampling west of the initial study area identified further contamination with the contaminant in this area primarily identified as perchloroethylene at levels ranging from 0.1−3.5 μg/m^3 in an area referred to as the Western study area. The study areas were then composed of a series of blocks combined to conform as closely to the areas of soil vapor contamination as possible. Cancer incident cases in residents at the time of diagnosis in the two areas were included in the study.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	No information.
>50% cohort with full latency	No information.
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study.
Blinded interviewers	

CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	Record study.
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	347 total cancers in males and females among an estimated population size of 3,540 (1980)−3,002 (2000).
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age and sex (ATSDR, 2006a). Age, sex, race (ATSDR, 2008). Medical record review of 15 kidney and 6 testicular cancer cases provided limited information on smoking, work history, and residential history for a small percentage of these cases (ATSDR, 2008).
Statistical methods	
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.8. *Studies in Arizona*

B.3.3.8.1. *Studies of West Central Phoenix Area, Maricopa County, AZ.*

B.3.3.8.1.1. ***Aickin et al. (1992), Aickin (2004).***

B.3.3.8.1.1.1. *Aickin et al. (1992) author's abstract.*

> Reports of a suspected cluster of childhood leukemia cases in West Central Phoenix have led to a number of epidemiological studies in the geographical area. We report here on a death certificate-based mortality study, which indicated an elevated rate ratio of 1.95 during 1966-1986, using the remainder of the Phoenix standard metropolitan statistical area (SMSA) as a comparison region. In the process of analyzing the data from this study, a methodology for dealing with denominator variability in a standardized mortality ratio was developed using a simple linear Poisson model. This new approach is seen as being of general use in the analysis of standardized rate ratios (SRR), as well as being particularly appropriate for cluster investigations.

B.3.3.8.1.1.2. *Aickin (2004) author's abstract.*

> BACKGROUND AND OBJECTIVES: Classical statistical inference has attained a dominant position in the expression and interpretation of empirical results in biomedicine. Although there have been critics of the methods of hypothesis testing, significance testing (P-values), and confidence intervals, these methods are used to the exclusion of all others. METHODS: An alternative metaphor and inferential computation based on credibility is offered here. RESULTS: It is illustrated in three datasets involving incidence rates, and its advantages over both classical frequentist inference and Bayesian inference, are detailed. CONCLUSION: The message is that for those who are unsatisfied with classical methods but cannot make the transition to Bayesianism, there is an alternative path.

B.3.3.8.1.1.3. *Study description and comment*. This study by staff of Arizona Department of Health Services of leukemia mortality or incidence rates among children $\leq$19 years old living at the time a death in West Central Phoenix in Maricopa County assume residence in the defined geographical area as a surrogate of undefined exposures. Aickin et al. (1994) adopted a classical statistical approach, linear Poisson regression, to estimate age-, sex- and calendar year adjusted relative risks for leukemia mortality between 1966 and 1986 among children 19 years of younger living in the study area at the time of death. Leukemia mortality rates for the rest of Maricopa County, excluding the study area and three additional geographic areas previously identified with hazardous waste contamination, were selected as the referent (Aickin et al., 1992). Aickin

(2004) adopt inferential or Bayesian approaches to test whether childhood leukemia incidence between 1966 and 1986 would confirm the mortality analysis observation.

Both studies use residence at time of diagnosis or death in the study area, West Central Phoenix, AZ, as the exposure surrogate; specific exposures such as drinking water contaminates are not examined nor is information on parental factors considered in the analysis. Some information on potential exposures in the community-at-large may be obtained from reports prepared by the AZ DHS of epidemiologic investigations of cancer mortality rates among residents of this area. Aickin et al. (1992) is the published finding on childhood leukemia. Past exposure to the population of West Central Phoenix to environmental contaminants has been difficult to quantify because of a paucity of environmental monitoring data (Flood et al., 1990). Community concerns about the environment focused on TCE found in drinking water in the late 1981, air pollution, from benzene emission from a nearby major gasoline storage and distribution facility, and pesticide residues. Two wells that occasionally supplemented the water supply in West Central Phoenix were closed after TCE was detected at the wellhead. The levels of TCE measured at the time contamination was detected were 8.9 ppb and 29.0 ppb (report does not identify the number of samples nor concentration ranges). The period over which contaminant water had been supplied from these wells was not known nor whether significant exposure to the population occurred after mixing with surface water. Other compounds identified in the contaminated plume besides TCE included 1,1-dichloroethylene, trans-1,2-dichloroethylene, chloroform, and chromium. The exposure assessment in the AZ DHS reports is inadequate to describe exposure potential to TCE to subjects of Aickin et al. (1992) and Aickin (2004). Moreover, potential etiologic factors for the observed elevated estimated relative risk for childhood leukemia bases are not examined. While these studies support an inference of elevated childhood leukemia rates in residents of West Central Phoenix, these studies provide little information on childhood leukemia and TCE exposure and contribute little weight in the overall weight-of-evidence analysis of cancer and TCE.

Aickin M, Chapin CA, Flood TJ, Englender SJ, Caldwell GG. 1992. Assessment of the spatial occurrence of childhood leukemia mortality using standardized rate ratios with a simple linear Poisson model. Int J Epidemiol 21:649−655.

Aickin M. 2004. Bayes without priors. J Clin Epidemiol 57:4−13.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Aickin et al. (1992) illustrated a methodologic approach to reduce variability in rate ratios from small-sized populations. Childhood leukemia mortality in a geographically-defined area in central Phoenix, AZ, was the case study adopted to illustrate methodologic approach. The analysis was not designed to examine possible etiologic factors. The purpose of Aickin (2004) "was to determine whether a 1.95 standardized mortality ratio [19] for leukemia in West Central Phoenix (compared to the remainder of Maricopa County) would be confirmed in an incidence study" [p. 8].
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Leukemia deaths among children ≤19 yrs of age between the years 1966 and 1986 and with addresses on death certificates in the geographically-defined study area were identified from Arizona death tapes. Referent group is childhood leukemia mortality rate of all other Maricopa residents excluding the study area and 3 other areas with identified hazardous waste contamination (Aickin et al., 1992). Incident cases of childhood leukemia (≤19 yrs) among residents living in study area were identified from the Arizona Cancer Registry and from cancer registry and medical record reviews at 13 area hospitals (Flood et al., 1990).
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer mortality (Aickin et al., 1992). Cancer incidence (Aickin, 2004).

Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	Mortality—ICD 7, ICDA 8, ICD 9 (Flood and Chapin, 1988). Incidence—ICD-O.
CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in geographical area is a surrogate of undefined exposures; possible exposures are not identified in the paper.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	38 childhood leukemia deaths over a period of 21 yrs. 49 childhood leukemia incident cases over a period of 21 yrs.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and year (1966−1969, 1979−1981, 1982−1986).
Statistical methods	Poisson regression using 1970, 1980, and 1985 population estimates from U.S. Bureau of the Census.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

B.3.3.8.2. *Studies in Tucson, Pima County, AZ.*

B.3.3.8.2.1. ***<u>Arizona Department of Health Services (AZ DHS, 1990, 1995)</u>***.

B.3.3.8.2.1.1. *Arizona Department of Health Services (AZ DHS, 1990) author's summary*.

> In 1986, responding to community concerns about possible past exposure to low levels of trichloroethylene in drinking water, a committee appointed by the Director of the Arizona Department of health Services recommended that the incidence of childhood leukemia and testicular cancer be studied in the population residing in the Tucson Airport Area (TAA). The study reported here was designed to count all cancer cases occurring in 0-19 year-old Pima County residents, and all testicular cancer cases in Pima County residents of all ages, during the 1970-1986 time period. Based on the incidence rates in the remainder of Pima County, approximately seven cases of childhood leukemia and approximately eight cases of testicular cancer would have been expected in the TAA. Eleven cases of leukemia (SIR = 1.50, 95% C.I. 0.76-2.70) and six cases of testicular cancer (SIR = 0.78, 95% C.I. 0.32-1.59) were observed. Statistical analyses showed that the incidence rates of these cancers were not significantly elevated. Additionally, it was determined that the rates of other childhood cancers in the TAA, grouped as lymphoma, brain/CNS and other, were not significantly elevated. The childhood leukemia, childhood cancer, and testicular cancer rates in Pima County were comparable to rates in other states and cities participating in the National Cancer Institute's Surveillance Epidemiology and End Results Program.

B.3.3.8.2.1.2. *Arizona Department of Health Services (AZ DHS, 1995) author's summary*.

> In 1986, responding to community concerns about possible past exposure to low levels of trichloroethylene in drinking water, a committee appointed by the Director of the Arizona Department of health Services recommended that the incidence of childhood leukemia and testicular cancer be studied in the population residing in the Tucson Airport Area (TAA). The study reported here was designed to count all cancer cases occurring in 0-19 year-old Pima County residents, and all testicular cancer cases in Pima County residents of all ages, during the 1986-1991 time period. Based on the incidence rates in the remainder of Pima County, approximately 3 cases of childhood leukemia and 4 cases of testicular cancer would have been expected in the TAA. Three cases of leukemia (SIR = .80; 95% C.I. 0.31-2.05) and 4 cases of testicular cancer (SIR = .93; 95% C.I. 0.37-2.35) were observed. Statistical analyses showed that the incidence rates of these cancers were not significantly elevated. Additionally, results indicate no statistically elevated incidence rates of childhood lymphoma, brain/CNS, and other childhood cancers, for ages 0-19, in the TAA. No consistent pattern of disease occurrence was observed when comparing the past incidence and mortality studies conducted by ADHS in the TAA with this present study regarding disease categories.

B.3.3.8.2.1.3. *Study description and comment*. These reports by staff of AZ DHS of cancer incidence among children ≤19 years old and of testicular cancer incidence among males living at the time a diagnosis in 1970−1986 or 1987−1991 in the Tucson International Airport Area (TAA) of southwest Tucson (AZ DHS, 1990, 1995) compared to incidence rates for the rest of Pima County were conducted in response to community concerns about cancer and possible past exposure to low levels of TCE in drinking water. In contrast to studies in West Central Phoenix, findings from the 1990 and 1995 AZ DHS studies in Tuscon have not been published in the peer-reviewed literature. Childhood cancers included were leukemia, brain/CSN, lymphoma, and a broad category of all other cancers diagnosed in children ≤19 years old. The Arizona Cancer Registry and reviews of medical records of 10 Pima county hospitals served as sources for identifying incident cases. The study area was defined as a geographical area overlaying a plume of contaminated groundwater and was comprised of five census tracts. The approximate areas boundaries are Ajo Way (north), Los Reales Road (south), Country Club Road (east), and the Santa Cruz River (west). Adjacent census tracts in Pima County were aggregated into four separate study areas and incident cancer rates during the 1970−1986 time period (AZ DHS, 1990) or 1987−1991 (AZ DHS, 1995) of the aggregated 4-area census tract, excluding the TAA area., were used to calculate expected numbers of cancers using the indirect standardization method and population estimates from 1960, 1970, 1975, 1980, and 1985 (AZ DHS, 1990) or 1990 (AZ DHS, 1995) of the U.S. Bureau of Census. A secondary analysis of AZ DHS (1990) compared the incidence rate of childhood leukemia and testicular cancer among Pima County residents to that reported to the SEER for a similar time period.

These studies assume residence in the defined geographical area as a surrogate of undefined exposures. The reports do not identify specific exposures for the individual subjects and some information on exposures in the community-at-large may be obtained from Public Health Assessments of the Tucson International Airport Area Superfund Site prepared by the AZ DHS for the ATSDR (2000, 2001). The TAA site includes one main contaminated groundwater plume with smaller areas of groundwater contamination located east of the main plume. Insufficient data existed to evaluate groundwater contamination prior to 1981. Studies conducted by AZ DHS in 1981−1982 showed TCE concentrations of above 5 ppb, the maximum contaminate level, in the main groundwater plume with TCE detected in some municipal drinking water wells at concentrations of up to 239 ppb. An ATSDR health assessment conducted in 1988 indicated that soil and groundwater in the Main Plume had been contaminated by chromium and volatile organic compounds such as TCE and dichloroethylene (DCE) (ATSDR, 2000). Sampling of private wells from 1981 through 1994 identified both drinking and irrigation private wells in and near the TIAA with TCE concentrations ranging from nondetect to

120 ppb. Concentrations of other VOCs and chromium from the 1980s are not presented in the ATSDR reports. Besides groundwater, areas of contaminated soil and sediment have also been identified as part of the site. The "Three Hangars" area of the airport was found to contain polychlorinated biphenyls in drainage areas with migration off-site into residential neighborhoods (ATSDR, 2001). The exposure assessment in these studies is inadequate to describe exposure to TCE. The studies provide little information on cancer risks and TCE exposure and carry little weight in the overall weight-of-evidence analysis.

AZ DHS (Arizona Department of Health Services). 1990. The incidence of childhood leukemia and testicular cancer in Pima County: 1970-1986. Prepared by the Arizona Department of Health Services, Division of Disease Prevention, Office of Risk Assessment and Investigation, Office of Chronic Disease Epidemiology. September 17, 1990.

AZ DHS (Arizona Department of Health Services). 1995. Update of the incidence of childhood leukemia and testicular cancer in Southwest Tucson, 1987-1991. Prepared by the Arizona Department of Health Services, Office of Risk Assessment and Investigation, Disease Prevention Services. June 6, 1995.

	Description
CATEGORY A: STUDY DESIGN	
Clear articulation of study objectives or hypothesis	Yes, from AZ DHS (1990), "1) To determine whether there was an elevated incidence of leukemia or other cancers among children residing in the Tucson Airport Area (TAA) and 2) To determine whether there was an elevated incidence of testicular cancer in males in the TAA." From AZ DHS (1995), "The objective of this study is to determine whether the incidence rates of childhood leukemia (ages 0-19) and testicular cancer in males of all ages were significantly elevated in the TAA when compared to the rest of Pima County for the years 1987 through 1991."
Selection and characterization in cohort studies of exposure and control groups and of cases and controls in case-control studies is adequate	Cases are identified from the Arizona Cancer Registry and review of medical records at 10 Pima County hospitals. The referent is incidence rates for the remaining population of Pima County, excluding the study area.
CATEGORY B: ENDPOINT MEASURED	
Levels of health outcome assessed	Cancer incidence.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma	ICD-O and ICD-9 or equivalent codes from ICDA-8, ICD-7, HICDA, or SNODO.

CATEGORY C: TCE-EXPOSURE CRITERIA	
Exposure assessment approach, including adoption of JEM and quantitative exposure estimates	Residence in geographical area is a surrogate of undefined exposures; possible exposures are not identified in the paper.
CATEGORY D: FOLLOW-UP (COHORT)	
More than 10% loss to follow-up	
>50% cohort with full latency	
CATEGORY E: INTERVIEW TYPE	
<90% face-to-face	Record study.
Blinded interviewers	
CATEGORY F: PROXY RESPONDENTS	
>10% proxy respondents	
CATEGORY G: SAMPLE SIZE	
Number of deaths in cohort mortality studies; numbers of total cancer incidence studies; numbers of exposed cases and prevalence of exposure in case-control studies	AZ DHS (1990), 31 childhood cancers—11 leukemia cases, 2 lymphoma, 3 CNS/Brain, and 15 other, and 6 testicular cancers. AZ DHS (1995), 11 childhood cancers—3 leukemia, 1 lymphoma, 2 CNS/Brain, and 5 other, and 4 testicular cancers.
CATEGORY H: ANALYSIS	
Control for potential confounders in statistical analysis	Age, sex, and year.
Statistical methods	SIRs calculated using indirect standardization.
Exposure-response analysis presented in published paper	No.
Documentation of results	Yes.

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APPENDIX C

Meta-Analysis of Cancer Results from Epidemiological Studies

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APPENDIX C. META-ANALYSIS OF CANCER RESULTS FROM EPIDEMIOLOGICAL STUDIES

C.1. METHODOLOGY

An initial review of the epidemiological studies indicated some evidence for associations between trichloroethylene (TCE) exposure and lymphomas and cancers of the kidney and liver. To investigate further these possible associations, we performed meta-analyses of the epidemiological study results for these three cancer types. Meta-analysis provides a systematic way to combine study results for a given effect across multiple (sufficiently similar) studies. The resulting summary (weighted average) estimate is a quantitatively objective way of reflecting results from multiple studies, rather than relying on a single study, for instance. Combining the results of smaller studies to obtain a summary estimate also increases the statistical power to observe an effect, if one exists. Furthermore, meta-analyses typically are accompanied by other analyses of the epidemiological studies, including analyses of publication bias and investigations of possible factors responsible for any heterogeneity across studies.

Given the diverse nature of the epidemiological studies for TCE, random-effects models were used for the primary analyses, and fixed-effect analyses were conducted for comparison. Both approaches combine study results (in this case, relative risk [RR] estimates) weighted by the inverse invariance; however, they differ in their underlying assumptions about what the study results represent and how the variances are calculated. For a random-effects model, it is assumed that there is true heterogeneity across studies and that both between-study and within-study components of variation need to be taken into account; this was done using the methodology of DerSimonian and Laird (1986). For a fixed-effect model, it is assumed that the studies are all essentially measuring the same thing and all the variance is within-study variance; thus, for the fixed-effect model, the RR estimate from each study is simply weighted by the inverse of the (within-study) variance of the estimate.

Studies for the meta-analyses were selected as described in Appendix B, Section II-9. The general approach for selecting RR estimates was to select the reported RR estimate that best reflected an RR for TCE exposure vs. no TCE exposure (overall effect). When available, RR estimates from internal analyses were selected over standardized incidence or mortality ratios (SIRs, SMRs) and adjusted RR estimates were generally selected over crude estimates. Incidence estimates would normally be preferred to mortality estimates; however, for the two studies providing both incidence and mortality results, incidence ascertainment was for a substantially shorter period of time than mortality follow-up, so the endpoint with the greater number of cases was used to reflect the results that had better case ascertainment. For separate analyses, an RR estimate for the highest exposure group was selected from studies that presented results for different exposure groups. Exposure groups based on some measure of cumulative exposure were preferred, if available; however, often duration was the sole exposure metric used. Specific selection choices are described in the following subsections detailing the actual analyses.

The meta-analysis calculations are based on (natural) logarithm-transformed values. Thus, each RR estimate was transformed to its natural logarithm (referred to here as "log RR," the conventional terminology in epidemiology), and either an estimate of the standard error (SE) of the log RR was obtained, from which to estimate the variance for the weights, or an estimate of the variance of the log RR was calculated directly. If the reported 95% confidence interval limits were proportionally symmetric about the observed RR estimate (i.e., upper confidence limit/RR ≈ RR/lower confidence limit), then an estimate of the SE of the log RR estimate was obtained using the formula

$$SE = \frac{\left[log\left(UCL\right) - log\left(LCL\right)\right]}{3.92}, \qquad \text{(Eq. C-1)}$$

where UCL is the upper confidence limit and LCL is the lower confidence limit (for 90% confidence intervals [CIs], the divisor is 3.29) (Rothman and Greenland, 1998). In all the TCE cohort studies reporting SMRs or SIRs as the overall RR estimates, reported CIs were calculated assuming the number of deaths (or cases) is approximately Poisson distributed. In such cases, the CIs are not proportionally symmetric about the RR estimate (unless the number of deaths is fairly large), and the SE of the log RR estimate was estimated as the inverse of the square root of the observed number of deaths (or cases) (Breslow and Day, 1987). In some case-control studies, no overall odds ratio (OR) was reported, so a crude OR estimate was calculated as OR = (a/b)/(c/d), where a, b, c, and d are the cell frequencies in a 2 × 2 table of cancer cases vs. TCE exposure, and the variance of the log OR was estimated using the formula

$$Var\left[log\left(OR\right)\right] = \frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}, \qquad \text{(Eq. C-2)}$$

in accordance with the method proposed by Woolf (1955), as described by Breslow and Day (1980).

The analyses that were performed for this assessment include

- meta-analyses to obtain overall summary estimates of RR
- heterogeneity analyses
- analyses of the influence of single studies on the summary estimates
- analyses of the sensitivity of the summary estimate to alternate study inclusion selections or to alternate selections of RR estimates from a study
- publication bias analyses
- meta-analyses to obtain summary estimates for the highest exposure groups in studies that provide data by exposure group, and
- consideration of some potential sources of heterogeneity across studies.

The analyses were conducted using Excel spreadsheets and the software package Comprehensive Meta-Analysis, Version 2 (© 2006, Biostat, Inc.). Figures were generated using the Comprehensive Meta-Analysis software. Note that for these figures, this software recalculates CIs for the studies based on the SE inputs, and the resulting CIs are not always identical to those reported in the original studies, in particular those based on Poisson distributions. However, the recalculated CIs are merely outputs and are not the basis for any calculations in the software; SEs were obtained as described above, and these SEs and the log RRs constitute the inputs for the meta-analysis calculations.

The heterogeneity (or homogeneity) analysis tests the hypothesis that the study results are homogeneous, i.e., that all the RR estimates are estimating the same population RR and the total variance is no more than would be expected from within-study variance. Heterogeneity was assessed using the statistic Q described by DerSimonian and Laird (1986). The Q-statistic represents the sum of the weighted squared differences between the summary RR estimate (obtained under the null hypothesis, i.e., using a fixed-effect model) and the RR estimate from each study, and, under the null hypothesis, Q approximately follows a χ^2 distribution with degrees of freedom equal to the number of studies minus one. However, this test can be under-powered when the number of studies is small, and it is only a significance test, i.e., it is not very informative about the *extent* of any heterogeneity. Therefore, the I^2 value (Higgins et al., 2003) was also considered. $I^2 = 100\% \times (Q - df)/Q$, where Q is the Q-statistic and df is the degrees of freedom, as described above. This value estimates the percentage of variation that is due to study heterogeneity. Typically, I^2 values of 25%, 50%, and 75% are considered low, moderate, and high amounts of heterogeneity, respectively.

Subgroup analyses were sometimes conducted to examine whether or not the combined RR estimate varied significantly between different types of studies (e.g., case-control vs. cohort studies). In such subgroup analyses of categorical variables (e.g., study design), analysis of variance was used to determine if there was significant heterogeneity between the subgroups. Applying analysis of variance to meta-analyses with two subgroups (df = 1), $Q_{\text{between subgroups}} = Q_{\text{overall}} - (Q_{\text{subgroup1}} + Q_{\text{subgroup2}}) = z\text{-value}^2$, where Q_{overall} is the Q-statistic calculated across all the studies and $Q_{\text{subgroup1}}$ and $Q_{\text{subgroup2}}$ are the Q-statistics calculated within each subgroup.

Publication bias is a systematic error that occurs if statistically significant studies are more likely to be submitted and published than nonsignificant studies. Studies are more likely to be statistically significant if they have large effect sizes (in this case, RR estimates); thus, an upward bias would result in a meta-analysis if the available published studies have higher effect sizes than the full set of studies that was actually conducted. One feature of publication bias is that smaller studies tend to have larger effect sizes than larger studies, since smaller studies need larger effect sizes in order to be statistically significant. Thus, many of the techniques used to analyze publication bias examine whether or not effect size is associated with study size. Methods used to investigate potential publication bias for this assessment included funnel plots, which plot effect size vs. study size (actually, SE vs. log RR here); the "trim and fill" procedure of Duvall and Tweedie (2000), which imputes the "missing" studies in a funnel plot (i.e., the studies needed to counterbalance an asymmetry in the funnel plot resulting from an ostensible publication bias) and recalculates a summary effect size with these studies present; forest plots (arrays of RRs and CIs by study) sorted by precision (i.e., SE) to see if effect size shifts with study size; Begg and Mazumdar rank correlation test (Begg and Mazumdar, 1994), which examines the correlation between effect size estimates and their variances after standardizing the effect sizes to stabilize the variances; Egger's linear regression test (Egger et al., 1997), which tests the significance of the bias reflected in the intercept of a regression of effect size/SE on 1/SE; and cumulative meta-analyses after sorting by precision to assess the impact on the summary effect size estimate of progressively adding the smaller studies.

C.2. META-ANALYSIS FOR LYMPHOMA

C.2.1. Overall Effect of TCE Exposure

C.2.1.1. *Selection of RR Estimates*

The selected RR estimates for lymphoma associated with TCE exposure from the selected epidemiological studies are presented in Table C-1 for cohort studies and in Table C-2 for case-control studies. A few of the more recent case-control studies classified lymphomas along the lines of the recent WHO/REAL classification system (World Health

Organization/Revised European-American Classification of Lymphoid Neoplasms) (Harris et al., 2000); however, most of the available TCE studies reported lymphoma results according to the International Classification of Diseases (ICD), Revisions 7, 8, and 9, and focused on non-Hodgkin lymphoma (NHL; ICD 200 + 202). For consistency of endpoint in the lymphoma meta-analyses, RR estimates for ICD 200 + 202 were selected, wherever possible; otherwise, estimates for the classification(s) best approximating NHL were selected. In addition, many of the studies provided RR estimates only for males and females combined, and we are not aware of any basis for a sex difference in the effects of TCE on lymphoma risk; thus, wherever possible, RR estimates for males and females combined were used. The only study of much size (in terms of number of lymphoma cancer cases) that provided results separately by sex was Raaschou-Nielsen (2003). This study reports an insignificantly higher SIR for females (1.4, 95% CI: 0.73, 2.34) than for males (1.2, 95% CI: 0.98, 1.52).

Beyond selecting adjusted RR estimates for lymphoma classification and both sexes, when multiple estimates were available, the preference was to select the RR estimate that represented the largest population in a study, while trying to minimize the likelihood of TCE exposure misclassification. Sensitivity analyses were generally done to investigate the impact of these alternate selection choices, as well as to estimate the impacts of study findings that were not reported.

Thus, for example, for Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only results were used in the primary analysis; however, an attempt was made to estimate the female contribution to an overall RR estimate for both sexes and its impact on the meta-analysis. Axelson et al. (1994) reported that there were no cases of lymphoma observed in females, but the expected number was not presented. To estimate the expected number, the expected number for males was multiplied by the ratio of female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for NHL.[1] The male results and the estimated female contribution were then combined into an RR estimate for both sexes assuming a Poisson distribution, and this alternate RR estimate for the Axelson et al. (1994) study was used in a sensitivity analysis.

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[1]Person-years for men and women ≤79 years were obtained from Axelson et al. (1994): 23516.5 and 3691.5, respectively. Lifetime age-adjusted incidence rates for NHL for men and women were obtained from the National Cancer Institute's 2000-2004 SEER-17 (Surveillance Epidemiology and End Results from 17 geographical areas) database (http://seer.cancer.gov/statfacts/html/nhl.html): 23.2/100,000 and 16.3/100,000, respectively. The calculation for estimating the expected number of cases in females in the cohort assumes that the males and females have similar TCE exposures and that the relative distributions of age-related incidence risk for the males and females in the cohort are adequately represented by the ratios of person-years and lifetime incidence rates used in the calculation.

Table C-1. Selected RR estimates for lymphoma associated with TCE exposure (overall effect) from cohort studies

Study	RR	95% LCL	95% UCL	RR type	log RR	SE(log RR)	Alternate RR estimates	Comments
Anttila et al., 1995	1.81	0.78	3.56	SIR	0.593	0.354	None	ICD-7 200 + 202.
Axelson et al., 1994	1.52	0.49	3.53	SIR	0.419	0.447	1.36 (0.44, 3.18) with estimated female contribution to SIR added (see text)	ICD-7 200 and 202. Results reported separately; combined assuming Poisson distribution. Results reported for males only, but there was a small female component to the cohort.
Boice et al., 1999	1.19	0.65	1.99	SMR	0.174	0.267	1.19 (0.83, 1.65) for any potential exposure	ICD-9 200 + 202. For potential routine exposure.
Greenland et al., 1994	0.76	0.24	2.42	OR	−0.274	0.590	None	ICD-8 200-202. Nested case-control study.
Hansen et al., 2001	3.1	1.3	6.1	SIR	1.13	0.354	None	ICD-7 200 + 202. Male and female results reported separately; combined assuming Poisson distribution.
Morgan et al., 1998	1.01	0.46	1.92	SMR	0.00995	0.333	1.36 (0.35, 5.21) unpublished RR for ICD 200 (see text)	ICD 200 + 202. Results reported by Mandel et al. (2006). ICD Revision 7, 8, or 9, depending on year of death.
Raaschou-Nielsen et al., 2003	1.24	1.01	1.52	SIR	0.215	0.104	None	ICD-7 200 + 202.
Radican et al., 2008	1.36	0.77	2.39	Mortality HR	0.307	0.289	None	ICD-8,-9 200 + 202; ICD-10 C82-C85. Time variable = age; covariates = sex and race. Referent group is workers with no chemical exposures.

Table C-1. Selected RR estimates for lymphoma associated with TCE exposure (overall effect) from cohort studies (continued)

Study	RR	95% LCL	95% UCL	RR type	log RR	SE(log RR)	Alternate RR estimates	Comments
Zhao et al., 2005	1.44	0.90	2.30	Mortality RR	0.363	0.239	Incidence RR: 0.77 (0.42, 1.39) Boice 2006 SMR for ICD-9 200 + 202: 0.21 (0.01, 1.18)	All lymphohematopoietic cancer (ICD-9 200-208), not just 200 + 202. Males only; adjusted for age, socioeconomic status (SES), time since first employment. Mortality results reflect more exposed cases (33) than do incidence results (17). Overall RR estimated by combining across exposure groups (see text). Boice 2006 cohort overlaps Zhao cohort; just 1 exposed death for ICD 200 + 202; 9 for 200−208 (vs. 33 in Zhao).

Table C-2. Selected RR estimates for lymphoma associated with TCE exposure from case-control studies[a]

Study	RR	95% LCL	95% UCL	log RR	SE(log RR)	Lymphoma type	Comments
Hardell et al., 1994	7.2	1.3	42	1.97	0.887	NHL	Rappaport classification system. Males only; controls matched for age, place of residence, vital status.
Miligi et al., 2006	0.93	--[b]	--[b]	−0.0726	0.168	NHL + CLL	NCI working formulation. Crude OR; overall adjusted OR not presented.
Nordstrom et al., 1998	1.5	0.7	3.3	0.405	0.396	HCL	HCL specifically. Males only; controls matched for age and county; analysis controlled for age.
Persson and Frederikson, 1999	1.2	0.5	2.4	0.182	0.400	NHL	Classification system not specified. Controls selected from same geographic areas; ORs stratified on age and sex.
Seidler et al., 2007	1.0	0.74	1.4	−0.223	0.177	B-cell and T-cell NHL	WHO classification. Overall results for B-cell and T-cell NHL from personal communication (see text). Adjusted for smoking and alcohol consumption. Case-control pairs matched on sex, region, and age.
Siemiatycki, 1991	1.1	0.5	2.5	0.0953	0.424	NHL	ICD-9 200 + 202. SE and 95% CI calculated from reported 90% CIs; males only; adjusted for age, income, and cigarette smoking index.
Wang et al., 2009	1.2	0.9	1.8	0.182	0.177	"NHL"; various lymphoma subtypes + mast cell tumors	ICD-O M-9590-9642, 9690-9701, 9740-9750. Females only; adjusted for age, family history of lymphohematopoietic cancers, alcohol consumption, and race.

[a]The RR estimates are all ORs for incident cases.
[b]Not calculated.

NHL: non-Hodgkin lymphoma; CLL: chronic lymphocytic leukemia; HCL: hairy cell leukemia (a subgroup of NHL).

Most of the selections in Tables C-1 and C-2 should be self-evident, but some are discussed in more detail here, in the order the studies are presented in the tables. For Boice et al. (1999), results for "potential routine exposure" were selected for the primary analysis, because this exposure category was considered to have less exposure misclassification, and results for "any potential exposure" were used in a sensitivity analysis. The Greenland et al. (1994) study is a case-control study nested within a worker cohort, and we treat it here as a cohort study (see Appendix B, Section II-9.1). For Morgan et al. (1998), the reported results did not allow for the combination of ICD 200 and 202, so the SMR estimate for the combined 200 + 202 grouping was taken from the meta-analysis paper of Mandel et al. (2006), who included one of the investigators from the Morgan et al. (1998) study. RR estimates for overall TCE exposure from internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (Environmental Health Strategies, 1997; the published paper only presented the internal analyses results for exposure subgroups), but only for ICD 200; from these, the RR estimate from the Cox model which included age and sex was selected, because those are the variables deemed to be important in the published paper (Morgan et al., 1998). Although the results from internal analyses are generally preferred, in this case the SMR estimate was used in the primary analysis and the internal analysis RR estimate was used in a sensitivity analysis because the latter estimate represented an appreciably smaller number of deaths (3, based on ICD 200 only) than the SMR estimate (9, based on ICD 200 + 202). For Radican et al. (2008), the Cox model hazard ratio (HR) from the 2000 follow-up was used. In the Radican et al. (2008) Cox regressions, age was the time variable, and sex and race were covariates. It should also be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE.

For Zhao et al. (2005), RR estimates were only reported for ICD-9 200−208 (all lymphohematopoietic cancers), and not for 200 + 202 alone. Given that other studies have not reported associations between leukemias and TCE exposure, combining all lymphohematopoietic cancers would dilute any lymphoma effect, and the Zhao results are expected to be an underestimate of any TCE effect on lymphoma alone. Another complication with the Zhao et al. (2005) study is that no results for an overall TCE effect are reported. We were unable to obtain any overall estimates from the study authors, so, as a best estimate, the results across the "medium" and "high" exposure groups were combined, under assumptions of group independence, even though the exposure groups are not independent (the "low" exposure group was the referent group in both cases). Zhao et al. (2005) present RR estimates for both incidence and mortality; however, the time frame for the incidence accrual is smaller than the time frame for mortality accrual and fewer exposed incident cases (17) were obtained than deaths (33). Thus, because better case ascertainment occurred for mortality than for incidence, the mortality results were used for the primary analysis, and the incidence results were used in a sensitivity analysis. A sensitivity analysis was also done using results from Boice et al. (2006) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies and should not be included in the meta-analysis concurrently. Boice et al. (2006) report an RR estimate for an overall TCE effect for lymphoma alone; however, it is based on far fewer cases (1 death in ICD-9 200 + 202; 9 deaths for 200−208) and is an SMR rather than an internal analysis RR estimate, so the Zhao et al. (2005) estimates are preferred for the primary analysis.

For the case-control studies, the main issue was the lymphoma classifications. Miligi et al. (2006) include chronic lymphocytic leukemias (CLLs) in their NHL results, consistent with the current WHO/REAL classification. Also, Miligi et al. (2006) do not report an overall adjusted RR estimate, so a crude estimate of the OR was calculated for the two TCE exposure categories together vs. no TCE exposure. The Nordstrom et al. (1998) study was a case-control study of hairy cell leukemias (HCLs), which are a subgroup of NHLs, so only results for HCL were reported. For Seidler et al. (2007), an overall adjusted OR for B-cell and T-cell NHL combined was kindly provided by Dr. Seidler (personal communication from Andreas Seidler, Bundesanstalt fur Arbeitsschutz u. Arbeitsmedizin, to Cheryl Scott, U.S. EPA, 13 November 2007). Wang et al. (2009) refer to their cases as "NHL" cases; however, according to the ICD-O classification system that they used, their cases are more specifically various particular subtypes of malignant lymphoma (9590-9642, 9690-9701) and mast cell tumors (9740-9750) (Morton et al., 2003). No alternate RR estimates were considered for any of the case-control studies of lymphoma.

C.2.1.2. *Results of Meta-Analyses*

Results from some of the meta-analyses that were conducted on the epidemiological studies of TCE and lymphoma are summarized in Table C-3. The summary estimate from the primary random effects meta-analysis of the 16 studies was 1.23 (95% CI: 1.04, 1.44) (see Figure C-1). No single study was overly influential; removal of individual studies resulted in summary, or "pooled," RR (RRp) estimates that ranged from 1.16 (with the removal of Hansen) to 1.28 (with the removal of Seidler) and were all statistically significant. Removal of Hardell, whose RR estimate is a relative outlier (see Figure C-1), only decreased the RRp estimate to 1.20 (1.04, 1.39), since this study does not contribute a lot of weight to the meta-analysis. Removal of studies other than Hansen or Hardell resulted in RRp estimates that were all greater than 1.20.

Table C-3. Summary of some meta-analysis results for TCE (overall) and lymphoma

Analysis	# of studies	Model	Summary RR estimate (RRp)	95% LCL	95% UCL	Heterogeneity	Comments
All studies	16	Random	1.23	1.04	1.44	Not significant (p = 0.10)	Statistical significance of RRp not dependent on individual studies.
		Fixed	1.19	1.06	1.34		
Cohort	9	Random	1.35	1.13	1.61	Not significant (p = 0.35)	Not significant difference between CC and cohort studies (p = 0.13).
		Fixed	1.33	1.14	1.54		Significant difference between CC and cohort studies (p = 0.03).
Case-control	7	Random	1.07	0.84	1.37	Not significant (p = 0.17)	
		Fixed	1.03	0.86	1.23		
Alternate RR selections[a]	16	Random	1.19	1.00	1.41	Not significant (p = 0.07)	With estimated Zhao overall RR for incidence rather than mortality.
	16	Random	1.21	1.01	1.45	Not significant (p = 0.053)	With Boice (2006) study rather than Zhao.
	16	Random	1.22	1.04	1.44	Not significant (p = 0.10)	With estimated female contribution to Axelson.
	16	Random	1.22	1.05	1.43	Not significant (p = 0.10)	With Boice (1999) any potential exposure SMR.
	16	Random	1.24	1.05	1.46	Not significant (p = 0.10)	With Morgan et al. (1998) unpublished RR.
Highest exposure groups	12	Random	1.57	1.27	1.94	None observable (fixed = random)	Statistical significance not dependent on single study. See Table C-5 for results with alternate RR selections.
		Fixed	1.57	1.27	1.94		

[a]Changing the primary analysis by one alternate RR each time; more details on alternate RR estimates in text.

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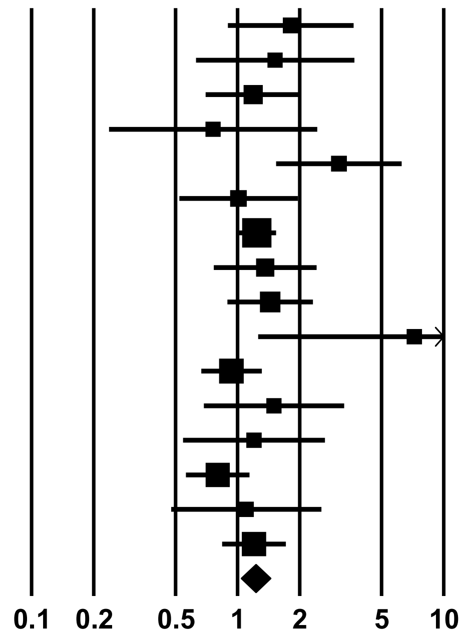
Study name	Statistics for each study				Rate ratio and 95% CI
	Rate ratio	Lower limit	Upper limit	p-Value	
Anttila 1995	1.810	0.905	3.619	0.093	
Axelson 1994	1.520	0.633	3.652	0.349	
Boice 1999	1.190	0.705	2.009	0.515	
Greenland 1994	0.760	0.239	2.413	0.642	
Hansen 2001	3.100	1.550	6.199	0.001	
Morgan 1998	1.010	0.526	1.941	0.976	
Raaschou-Nielsen 2003	1.240	1.011	1.521	0.039	
Radican 2008	1.360	0.772	2.396	0.287	
Zhao 2005 mort	1.437	0.899	2.297	0.130	
Hardell 1994	7.200	1.267	40.923	0.026	
Miligi 2006	0.933	0.671	1.298	0.682	
Nordstrom 1998	1.500	0.691	3.257	0.305	
Persson&Fredrikson 1999	1.200	0.548	2.629	0.649	
Seidler 2007	0.800	0.566	1.131	0.207	
Siemiatycki 1991	1.100	0.479	2.525	0.822	
Wang 2008	1.200	0.849	1.697	0.302	
	1.228	1.044	1.444	0.013	



random effects model

Figure C-1. Meta-analysis of lymphoma and overall TCE exposure. The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the summary RR estimate, and the horizontal extremes depict the 95% CI limits.

Similarly, the RRp estimate was not highly sensitive to alternate RR estimate selections. Use of the five alternate selections, individually, resulted in RRp estimates that ranged from 1.19 to 1.24 (see Table C-3) and were all statistically significant except when the Zhao incidence estimate (p = 0.050) was used instead of the Zhao mortality estimate. As discussed above, the Zhao mortality estimate is preferred over the incidence estimate in this instance because it is based on nearly twice as many cases (33 vs. 17).

There was some heterogeneity apparent across the 16 studies, although it was not statistically significant ($p = 0.10$). The I^2 value (see Section C.1) was 33%, suggesting low-to-moderate heterogeneity. Subgroup analyses were done examining the cohort and case-control studies separately. With the random effects model (and tau-squared not pooled across subgroups), the resulting RRp estimates were 1.35 (95% CI: 1.13, 1.61) for the cohort studies and 1.07 (0.84, 1.37) for the case-control studies. There was residual heterogeneity in each of the subgroups, but in neither case was it statistically. I^2 values were 10% for the cohort studies, suggesting low heterogeneity, and 33% for the case-control studies, suggesting low-to-moderate heterogeneity. The difference between the RRp estimates for the cohort and case-control subgroups was not statistically significant under the random effects model, although it was under the fixed effect model (see Table C-3). Some thought was given to further analyses to investigate the source(s) of the heterogeneity, such as qualitative tiering or subgroups based on likelihood for correct exposure classification or on likelihood for higher vs. lower exposures across the studies. Ultimately, these approaches were rejected because in many of the studies it was difficult to judge (and weight) the extent of exposure misclassification or the degree of TCE exposure with any precision. In other words, there was inadequate information to reliably assess either the extent to which each study accurately classified exposure status or the relative TCE exposure levels and prevalences of exposure to different levels across studies. See Section C.2.3 below for a qualitative discussion of some potential sources of heterogeneity.

As discussed in Section C.1, publication bias was examined in several different ways. The funnel plot in Figure C-2 suggests some relationship between RR estimate and study size (if there were no relationship, the studies would be symmetrically distributed around the pooled RR estimate rather than veering towards higher RR estimates with increasing SEs), although the observed asymmetry is highly influenced by the Hardell study, which is a relative outlier and which contributes little weight to the overall meta-analysis, as discussed above. The Begg and Mazumdar rank correlation test and Egger's linear regression test were not statistically significant; it should be noted, however, that both of these tests have low power. Duval and Tweedie's trim-and-fill procedure yielded a pooled RR estimate (under the random effects model) of 1.13 (95% CI: 0.94, 1.35) when the 4 studies deemed missing from the funnel plot were filled into the meta-analysis (these studies are filled in so as to counter-balance the apparent asymmetry of the more extreme values in the funnel plot). Eliminating the Hardell study made little difference to the results of the publication bias analyses. The results of a cumulative meta-analysis, incorporating studies with increasing SE one at a time, are depicted in Figure C-3. This procedure is a transparent way of examining the effects of including studies with increasing SE. The figure shows that the pooled RR estimate is 1.05 after inclusion of the 4 largest (i.e., most precise) studies, which constitute about 50% of the weight. The pooled RR estimate increases to 1.12 with inclusion of the 8 most precise studies, which represent ½ of the total number of studies and about 75% of the weight. The pooled RR estimate becomes fairly stable after addition of the next 2 most precise study (RRp = 1.21), which adds another 9% of the weight. Adding in the 6 least precise studies (16% of the weight) barely increases the pooled RR estimate further. In summary, there is some evidence of potential publication bias in this data set. It is uncertain, however, that this reflects actual publication bias rather than an association between effect size and SE resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias in this data set, it does not appear to account completely for the findings of an increased lymphoma risk.

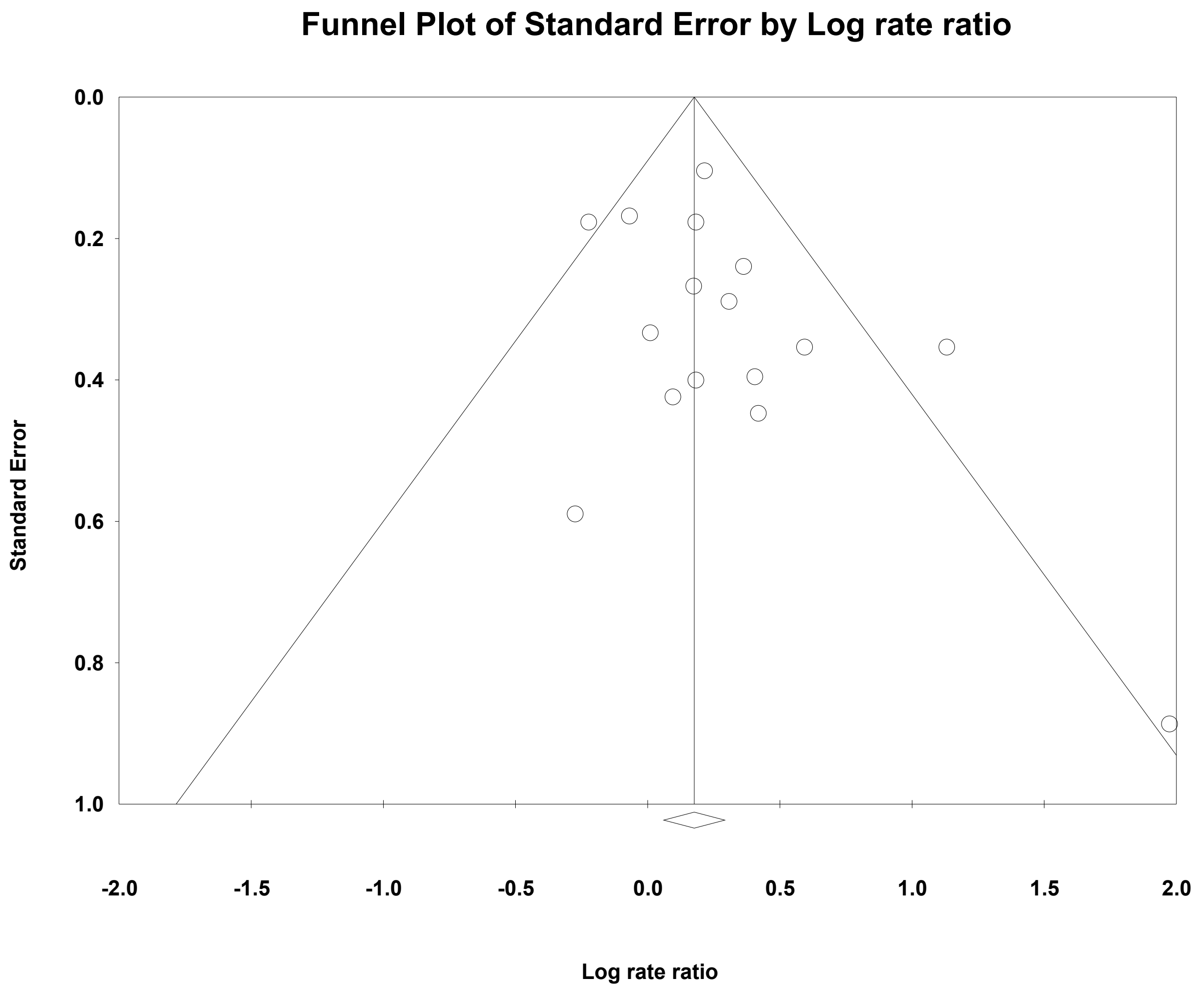


Figure C-2. Funnel plot of SE by log RR estimate for TCE and lymphoma studies.

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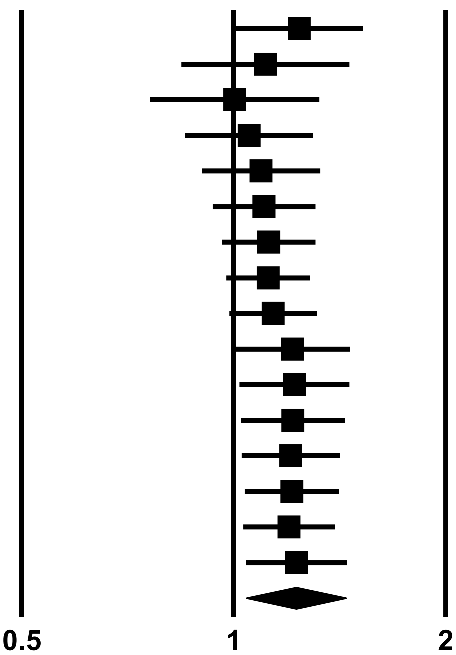
Study name	Point	Lower limit	Upper limit	p-Value
Raaschou-Nielsen 2003	1.240	1.011	1.521	0.039
Miligi 2006	1.109	0.845	1.456	0.455
Seidler 2007	1.004	0.763	1.320	0.980
Wang 2008	1.052	0.855	1.294	0.631
Zhao 2005 mort	1.094	0.904	1.324	0.356
Boice 1999	1.105	0.936	1.303	0.238
Radican 2008	1.122	0.965	1.304	0.135
Morgan 1998	1.120	0.979	1.281	0.099
Anttila 1995	1.138	0.989	1.310	0.071
Hansen 2001	1.212	1.006	1.459	0.043
Nordstrom 1998	1.220	1.022	1.456	0.028
Persson&Fredrikson 1999	1.214	1.027	1.434	0.023
Siemiatycki 1991	1.206	1.029	1.412	0.020
Axelson 1994	1.210	1.040	1.408	0.014
Greenland 1994	1.199	1.035	1.390	0.016
Hardell 1994	1.228	1.044	1.444	0.013
	1.228	1.044	1.444	0.013

Cumulative rate ratio (95% CI)



random effects model; cumulative analysis, sorted by SE

Figure C-3. Cumulative meta-analysis of TCE and lymphoma studies, progressively including studies with increasing SEs.

C.2.2. Lymphoma Effect in the Highest Exposure Groups

C.2.2.1. *Selection of RR Estimates*

The selected RR estimates for lymphoma in the highest TCE exposure categories, for studies that provided such estimates, are presented in Table C-4. All 8 cohort studies (but not the nested case-control study of Greenland et al. [1994]) and 4 of the 7 case-control studies did report lymphoma risk estimates categorized by exposure level. As in Section C.2.1.1 for the overall risk estimates, estimates to best correspond to NHL as represented by ICD-7, -8, and -

9 200 and 202 were selected, and, wherever possible, RR estimates for males and females combined were used.

As above for the overall TCE effect, for Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only high-exposure group results were used in the primary analysis; however, an attempt was made to estimate the female contribution to a high-exposure group RR estimate for both sexes and its impact on the meta-analysis. To estimate the expected number in the highest exposure group for females, the expected number in the highest exposure group for males was multiplied by the ratio of total female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for NHL. The RR estimate for both sexes was used as an alternate RR estimate for the Axelson et al. (1994) study in a sensitivity analysis.

For Boice et al. (1999), only results for workers with "any potential exposure" (rather than "potential routine exposure") were presented by exposure category, and the referent group is workers not exposed to any solvent. For Hansen et al. (2001), exposure group data were presented only for males. To estimate the female contribution to a highest-exposure group RR estimate for both sexes, it was assumed that the expected number of cases in females had the same overall-to-highest-exposure group ratio as in males. The RR estimate for both sexes was then calculated assuming a Poisson distribution, and this estimate was used in the primary analysis. Hansen et al. (2001) present results for three exposure metrics; the cumulative exposure metric was preferred for the primary analysis, and results for the other two metrics were used in sensitivity analyses. For Morgan et al. (1998), results did not allow for the combination of ICD 200 and 202, so the highest-exposure group RR estimate for ICD 200 only was used. The primary analysis used results for the cumulative exposure metric, and a sensitivity analysis was done with the results for the peak exposure metric.

For Radican et al. (2008), it should be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE. In addition, exposure group results were reported separately for males and females and were combined for this assessment using inverse-variance weighting, as in a fixed effect meta-analysis. Radican et al. (2008) present only mortality HR estimates by exposure group; however, in an earlier follow-up of this same cohort, Blair et al. (1998) present both incidence and mortality RR estimates by exposure group. The mortality RR estimate based on the more recent follow-up of Radican et al. (2008) (17 deaths in the highest exposure group) was used in the primary analysis, while the incidence RR estimate based on similarly combined results from Blair et al. (1998) (9 cases) was used as an alternate estimate in a sensitivity analysis.

Table C-4. Selected RR estimates for lymphoma risk in highest TCE exposure groups

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE(log RR)	Alternate RR estimates	Comments
Anttila et al., 1995	1.4	0.17	5.04	100+ µmol/L U-TCA[a]	0.336	0.707	none	SIR. ICD 200 + 202.
Axelson et al., 1994	6.25	0.16	34.83	≥2-yr exposure and 100+ mg/L U-TCA	1.83	1.00	5.62 (0.14, 31.3) with estimated female contribution added (see text)	SIR. ICD 200 + 202. Results reported for males only, but there was a small female component to the cohort.
Boice et al., 1999	1.62	0.82	3.22	≥5-yr exposure	0.482	0.349	None	Mortality RR. ICD 200 + 202. For potential routine or intermittent exposure. Adjusted for date of birth, dates 1st and last employed, race, and sex. Referent group is workers not exposed to any solvent.
Hansen et al., 2001	2.7	0.56	8.0	≥1080 mos × mg/m^3	0.993	0.577	3.7 (1.0, 9.5) for ≥75 mos exposure duration 2.9 (0.79, 7.5) for ≥19 mg/m^3 mean exposure	SIR. ICD 200 + 202. Exposure-group results presented only for males. Female results estimated and combined with male results assuming Poisson distribution (see text).
Morgan et al., 1998	0.81	0.1	6.49	High cumulative exp. score	−0.211	1.06	1.31 (0.28, 6.08) for med/high peak vs. low/no	Mortality RR. ICD 200 only. Adjusted for age and sex.
Raaschou-Nielsen et al., 2003	1.6	1.1	2.2	≥5 yrs in subcohort with expected higher exp. levels	0.470	0.183	None	SIR. ICD 200 + 202.

Table C-4. Selected RR estimates for lymphoma risk in highest TCE exposure groups (continued)

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE(log RR)	Alternate RR estimates	Comments
Radican et al., 2008	1.41	0.71	2.81	>25 unit-yr	0.337	0.350	Blair et al. (1998) 0.97 (0.42, 2.2) incidence RR	Mortality HR. ICD 200 + 202. Male and female results presented separately and combined (see text). Cox regression time variable = age; covariate = race. Referent group is workers with no chemical exposures.
Zhao et al., 2005	1.30	0.52	3.23	High exposure score	0.262	0.466	Incidence RR: 0.20 (0.03, 1.46)	Mortality RR. Results for all lymphohematopoietic cancer (ICD-9 200−208), not just 200 + 202. Males only; adjusted for age, SES, time since first employment. Mortality results reflect more exposed cases (6 in high-exposure group) than do incidence results (1 in high-exposure group).
Miligi et al., 2006	1.2	0.7	2.0	Med/high exposure intensity	0.182	0.268	1.0 (0.5, 2.6) for med/high intensity and >15-yr exp.	Incidence OR. NHL + CLL (see Section C.2.1.1).
Seidler et al., 2007	2.3	1.0	5.2	>35 ppm-yr	0.833	0.421	None	Incidence OR. Results for B-cell and T-cell NHL from personal communication (see Section C.2.1.1). Adjusted for smoking and alcohol consumption. Case-control pairs matched on sex, region, and age.
Siemiatycki 1991	0.8	0.2	3.3	Substantial	−0.223	0.719	None	Incidence OR. NHL. SE and 95% CI calculated from reported 90% CIs. Males only; adjusted for age, income, and cigarette smoking index.
Wang et al., 2009	2.2	0.9	5.4	Medium-high intensity	0.788	0.457	None	Incidence OR. "NHL" (various malignant lymphoma subtypes and mast cell tumors). Females only; adjusted for age, family history of lymphohematopoietic cancers, alcohol consumption, and race.

[a]Mean personal trichloroacetic acid in urine. 1 µmol/L = 0.1634 mg/L.

For Zhao et al. (2005), RR estimates were only reported for ICD-9 200−208 (all lymphohematopoietic cancers), and not for 200 + 202 alone. Given that other studies have not reported associations between leukemias and TCE exposure, combining all lymphohematopoietic cancers would dilute any lymphoma effect, and the Zhao results are expected to be an underestimate of any TCE effect on lymphoma alone. Zhao et al. (2005) present RR estimates for both incidence and mortality in the highest exposure group; however, the time frame for the incidence accrual is smaller than the time frame for mortality accrual and fewer incident cases (1) were obtained than deaths (6), so the mortality results were used for the primary analysis to reflect the better case ascertainment in the mortality data, and the incidence results were used in a sensitivity analysis.

Miligi et al. (2006) include CLLs in their NHL results, consistent with the current WHO/REAL classification. Miligi et al. (2006) report RR estimates for medium and high exposure intensity overall and by duration of exposure; however, there was incomplete information for the duration breakdowns (e.g., a case missing), so the RR estimate for med/high exposure intensity overall was used in the primary analysis, and the RR estimate for med/high exposure for >15 years was used in a sensitivity analysis. For Seidler et al. (2007), an adjusted OR for B-cell and T-cell NHL combined for the >35 ppm-years exposure category was kindly provided by Dr. Seidler (personal communication from Andreas Seidler, Bundesanstalt fur Arbeitsschutz u. Arbeitsmedizin, to Cheryl Scott, U.S. EPA, 13 November 2007). Wang et al. (2009) refer to their cases as "NHL" cases; however, according to the ICD-O classification system that they used, their cases are more specifically various particular subtypes of malignant lymphoma (9590-9642, 9690-9701) and mast cell tumors (9740-9750) (Morton et al., 2003).

C.2.2.2. *Results of Meta-Analyses*

Results from the meta-analyses that were conducted for lymphoma in the highest exposure groups are summarized at the bottom of Table C-3 and reported in more detail in Table C-5. The pooled RR estimate from the primary random effects meta-analysis of the 12 studies with results presented for exposure groups was 1.57 (95% CI: 1.27, 1.94) (see Figure C-4). No single study was overly influential; removal of individual studies resulted in RRp estimates that were all statistically significant (all with $p \leq 0.001$) and that ranged from 1.53 (with the removal of Seidler) to 1.65 (with the removal of Miligi). Similarly, the RRp estimate was not highly sensitive to alternate RR estimate selections. Use of the 7 alternate selections, individually, resulted in RRp estimates that were all statistically significant (all with $p < 0.001$) and all in the narrow range from 1.54 to 1.60 (see Table C-5). There was no observable heterogeneity across the 12 studies in either the primary analysis or any of the alternate RR analyses.

Table C-5. Summary of some meta-analysis results for TCE (highest exposure groups) and lymphoma

Analysis	Model	Combined RR estimate	95% LCL	95% UCL	Heterogeneity	Comments
Primary analysis	Random	1.57	1.27	1.94	None obs (fixed = random)	Statistical significance not dependent on single study.
Alternate RR selections[a]	Random	1.54	1.24	1.91	None obs	With Blair et al. (1998) incidence RR instead of Radican mortality HR.
	Random	1.55	1.24	1.92	None obs	With Zhao incidence.
	Random	1.57	1.27	1.94	None obs	With estimated female contribution for Axelson.
	Random	1.57	1.27	1.95	None obs	With Morgan peak.
	Random	1.58	1.28	1.96	None obs	With Hansen mean exposure.
	Random	1.60	1.28	2.00	None obs	With Miligi with >15 yrs.
	Random	1.60	1.30	1.98	None obs	With Hansen duration.

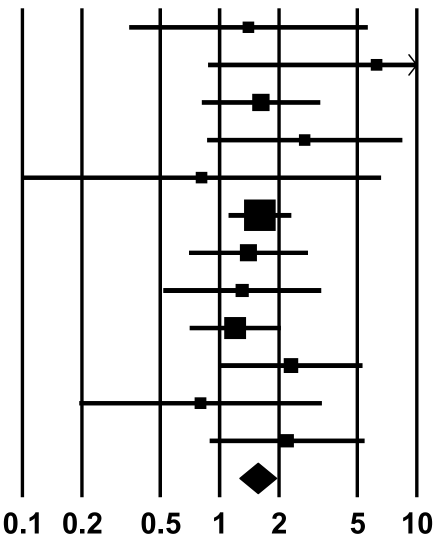
[a]Changing the primary analysis by one alternate RR estimate each time.

obs = observable.

TCE and Lymphoma - highest exposure groups

Study name	Rate ratio	Lower limit	Upper limit	p-Value
Anttila 1995	1.400	0.350	5.598	0.634
Axelson 1994	6.250	0.880	44.369	0.067
Boice 1999	1.620	0.818	3.210	0.167
Hansen 2001 cum exp	2.700	0.871	8.372	0.085
Morgan 1998	0.810	0.101	6.525	0.843
Raaschou-Nielsen 2003	1.600	1.119	2.288	0.010
Radican 2008 mort	1.400	0.705	2.780	0.336
Zhao 2005 mort	1.300	0.522	3.240	0.573
Miligi 2006	1.200	0.709	2.028	0.497
Seidler 2007	2.300	1.008	5.250	0.048
Siemiatycki 1991	0.800	0.195	3.275	0.756
Wang 2009	2.199	0.898	5.385	0.085
	1.569	1.267	1.942	0.000

Rate ratio and 95% CI



random effects model; same for fixed

Figure C-4. Meta-analysis of lymphoma and TCE exposure—highest exposure groups. (The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the pooled RR estimate, and the horizontal extremes depict the 95% CI limits.)

C.2.3. Discussion of Lymphoma Meta-Analysis Results

For the most part, the meta-analyses of the overall effect of TCE exposure on lymphoma suggest a small, statistically significant increase in risk. The pooled estimate from the primary random effects meta-analysis of the 16 studies was 1.23 (95% CI: 1.04, 1.44). This result was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. In terms of the statistical significance of the RRp estimate, the only alternate analysis (involving either a study removal or an alternate RR estimate) that did not yield a statistically significant RRp was the analysis in which the Zhao mortality RR estimate was substituted with the incidence estimate, resulting in an RRp estimate of 1.19 (1.00, 1.41); although, as noted above, this substitution is considered clearly inferior to the Zhao mortality estimate that was used in the primary analysis. Thus, the finding of an increased risk of lymphoma associated with TCE exposure, though the increased risk is not large in magnitude, is fairly robust.

There is some evidence of potential publication bias in this data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias in this data set, it does not appear to account completely for the finding of an increased lymphoma risk.

Although there was some heterogeneity across the 16 studies, it was not statistically significant ($p = 0.10$). The I^2 value was 33%, suggesting low-to-moderate heterogeneity. Similarly, when subgroup analyses were done of cohort and case-control studies separately, there was some observable heterogeneity in each of the subgroups, but it was not statistically significant in either case. I^2 values were 10% for the cohort studies, suggesting low heterogeneity, and 33% for the case-control studies, suggesting low-to-moderate heterogeneity. In the subgroup analyses, the increased risk of lymphoma was strengthened in the cohort study analysis and virtually eliminated in the case-control study analysis, although the subgroup RRp estimates were not statistically significantly different under the random effects model. Study design itself is unlikely to be an underlying cause of heterogeneity and, to the extent that it may explain some of the differences across studies, is more probably a surrogate for some other difference(s) across studies that may be associated with study design. Furthermore, other potential sources of heterogeneity may be masked by the broad study design subgroupings. The true source(s) of heterogeneity across these studies is an uncertainty. As discussed above, further quantitative investigations of heterogeneity were ruled out because of database limitations. A qualitative discussion of some potential sources of heterogeneity follows.

Study differences in exposure assessment approach, exposure prevalence, average exposure intensity, and lymphoma classification are possible sources of heterogeneity. Many studies included TCE assignment from information on job and task exposures, e.g., a job-exposure matrix (JEM) (Siemiatycki, 1991; Morgan et al., 1998; Boice et al., 1999, 2006; Zhao et al., 2005; Miligi et al., 2006; Seidler et al., 2007; Radican et al., 2008; Wang et al., 2009), or from an exposure biomarker in either breath or urine (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001). Three case-control studies relied on self-reported exposure to TCE (Hardell et al., 1994; Nordstrom et al., 1998; Persson and Fredrikson, 1999). Misclassification is possible with all exposure assessment approaches. No information is available to judge the degree of possible misclassification bias associated with a particular exposure assessment approach; it is quite possible that in some cohort studies, in which past exposure is inferred from various data sources, exposure misclassification may be as great as in population-based or hospital-based case-control studies. Approaches based upon JEMs can provide order-of-magnitude estimates that are useful for distinguishing groups of workers with large differences in exposure; however, smaller differences usually cannot be reliably distinguished (NRC, 2006). Biomonitoring can provide information on potential TCE exposure in an individual, but the biomarkers used aren't necessarily specific for TCE and they reflect only recent exposures. The lack of heterogeneity in the analysis of the highest exposure groups provides some evidence of exposure misclassification as a source of heterogeneity in the overall analysis.

General population studies have special problems in evaluating exposure, because the subjects could have worked in any job or setting that is present within the population (Copeland et al., 1977; Nelson et al., 1994; McGuire et al., 1998; 't Mannetje et al., 2002; NRC, 2006). Low exposure prevalence in the four population case-control studies (Siemiatycki, 1991; Miligi et al., 2006; Seidler et al., 2007; Wang et al., 2009) may be another source of heterogeneity. Prevalence of TCE exposure among cases in the case-control studies was low, ranging from 3% in Siemiatycki (1991) to 13% in Seidler et al. (2007) and Wang et al. (2009). However, prevalence of high TCE exposure in these case-control studies was even rarer—3% of all cases in Miligi et al. (2006) and Seidler et al. (2007), 2% in Wang et al. (2009), and less than 1% in Siemiatycki (1991). Low exposure prevalence, especially in the relatively large Miligi et al. (2006) and Seidler et al. (2007) case-control studies (see Figure C-1), may be one of the underlying characteristics differentiating the case-control and cohort studies and explaining some of the heterogeneity across the studies.

Study differences in lymphoma groupings and in lymphoma classification schemes are another potential source of heterogeneity in the meta-analysis. All studies included a broad but sometimes slightly different group of lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms, with the exception of the Nordstrom et al. (1998) case-control study, which examined hairy cell leukemia, now considered a lymphoma, and the Zhao et al. (2005) cohort study, which reported only results for *all* lymphohematopoietic cancers, including nonlymphoid types. Persson and Fredrikson (1999) do not identify the classification system for defining NHL, and Hardell et al. (1994) define NHL using the Rappaport classification system. Miligi et al. (2006) used an NCI classification system and considered chronic lymphocytic leukemias and NHLs together as lymphomas, while Seidler et al. (2007) used the REAL classification system, which reclassifies lymphocytic leukemias and NHLs as lymphomas of B-cell or T-cell origin. The cohort studies (except for Zhao et al.) and the case-control study of Siemiatycki (1991) have some consistency in coding NHL, with NHL defined as lymphosarcoma and reticulum-cell sarcoma (ICD code 200) and other lymphoid tissue neoplasms (ICD 202) using the ICD Revisions 7, 8, or 9. Revisions 7 and 8 are essentially the same with respect to NHL; under Revision 9, the definition of NHL was broadened to include some neoplasms previously classified as Hodgkin's lymphomas (Banks, 1992). Wang et al. (2009) refer to their cases as "NHL" cases; however, according to the ICD-O classification system that they used, their cases are more specifically various particular subtypes of malignant lymphoma (9590-9642, 9690-9701) and mast cell tumors (9740-9750) (Morton et al., 2003).

Twelve of the 16 studies categorized results by exposure level. Different exposure metrics were used, and the purpose of combining results across the different highest exposure groups was not to estimate an RRp associated with some level of exposure, but rather to see the impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of lymphoma, the effects should be more apparent in the highest exposure groups. Indeed, the RRp estimate from the primary meta-analysis of the highest exposure group results was 1.57 (95% CI: 1.27, 1.94), which is greater than the RRp estimate of 1.23 (95% CI: 1.04, 1.44) from the overall exposure analysis. This result for the highest exposure groups was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. Heterogeneity was not observed in any of the relevant analyses. The robustness of this finding lends substantial support to a conclusion that TCE exposure increases the risk of lymphoma.

C.3. META-ANALYSIS FOR KIDNEY CANCER

C.3.1. Overall Effect of TCE Exposure

C.3.1.1. *Selection of RR Estimates*

The selected RR estimates for kidney cancer associated with TCE exposure from the epidemiological studies are presented in Table C-6 for cohort studies and in Table C-7 for case-control studies. The majority of the cohort studies reported results for all kidney cancers, including cancers of the renal pelvis and ureter (i.e., ICD-7 180; ICD-8 and -9 189.0−189.2; ICD-10 C64−C66); whereas the majority of the case-control studies focused on renal cell carcinoma (RCC), which comprises roughly 85% of kidney cancers. Where both all kidney cancer and RCC were reported, the primary analysis used the results for RCC, because RCC and the other forms of kidney cancer are very different cancer types and it seemed preferable not to combine them; the results for all kidney cancers were then used in a sensitivity analysis. The preference for the RRC results alone is supported by the results in rodent cancer bioassays, where TCE-associated rat kidney tumors are observed in the renal tubular cells (Section 4.3.5), and in metabolism studies, where the focus of studies for the GSH conjugation pathway (considered the primary metabolic pathway for kidney toxicity) is in renal cortical and tubular cells (Sections 3.3.3.2 and 4.3.6).

As for lymphoma, many of the studies provided RR estimates only for males and females combined, and we are not aware of any basis for a sex difference in the effects of TCE on kidney cancer risk; thus, wherever possible, RR estimates for males and females combined were used. Of the three larger (in terms of number of cases) studies that did provide results separately by sex, Dosemeci et al. (1999) suggest that there may be a sex difference for TCE exposure and RCC (OR = 1.04 [95% CI: 0.6, 1.7] in males and 1.96 [1.0, 4.0] in females), while Raaschou-Nielsen et al. (2003) report the same SIR (1.2) for both sexes and crude ORs calculated from data from the Pesch et al. (2000) study (provided in a personal communication from Baeta Pesch, Forschungsinstitut für Arbeitsmedizin (BGFA), to Cheryl Scott, U.S. EPA, 21 February 2008) are 1.28 for males and 1.23 for females. Radican et al. (2008) and Hansen et al. (2001) also present some results by sex, but both of these studies have too few cases to be informative about a sex difference for kidney cancer.

Most of the selections in Tables C-6 and C-7 should be self-evident, but some are discussed in more detail here, in the order the studies are presented in the tables. For Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only results were used in the primary analysis; however, as for lymphoma, an attempt was made to estimate the female contribution to an overall RR estimate for both sexes and its impact on the meta-analysis. Axelson et al. (1994) reported neither the observed nor the expected number of kidney cancer cases for females. It was assumed that none were observed. To estimate the expected number, the expected number for males was multiplied by the ratio of female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for kidney cancer.[2] The male results and the estimated female contribution were then combined into an RR estimate for both sexes assuming a Poisson distribution, and this alternate RR estimate for the Axelson et al. (1994) study was used in a sensitivity analysis.

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[2]Person-years for men and women ≤79 years were obtained from Axelson et al. (1994): 23516.5 and 3691.5, respectively. Lifetime age-adjusted incidence rates for cancer of the kidney and renal pelvis for men and women were obtained from the National Cancer Institute's 2000–2004 SEER-17 (Surveillance Epidemiology and End Results from 17 geographical locations) database (http://seer.cancer.gov/statfacts/html/kidrp.html): 17.8/100,000 and 8.8/100,000, respectively. The calculation for estimating the expected number of cases in females in the cohort assumes that the males and females have similar TCE exposures and that the relative distributions of age-related incidence risk for the males and females in the cohort are adequately represented by the ratios of person-years and lifetime incidence rates used in the calculation.

Table C-6. Selected RR estimates for kidney cancer associated with TCE exposure (overall effect) from cohort studies

Study	RR	95% LCL	95% UCL	RR type	log RR	SE(log RR)	Alternate RR estimates	Comments
Anttila et al., 1995	0.87	0.32	1.89	SIR	−0.139	0.408	none	ICD-7 180.
Axelson et al., 1994	1.16	0.42	2.52	SIR	0.148	0.408	1.07 (0.39, 2.33) with estimated female contribution to SIR added (see text)	ICD-7 180. Results reported for males only, but there was a small female component to the cohort.
Boice et al., 1999	0.99	0.4	2.04	SMR	−0.010	0.378	None	ICD-9 189.0−189.2. For potential routine exposure. Results for any potential exposure not reported.
Greenland et al., 1994	0.99	0.30	3.32	OR	−0.010	0.613	None	Nested case-control study. ICD-8 codes not specified, presumably all of 189.
Hansen et al., 2001	1.1	0.3	2.8	SIR	0.095	0.500	None	ICD-7 180. Male and female results reported separately; combined assuming Poisson distribution.
Morgan et al., 1998	1.14	0.51	2.58	Mortality RR	0.134	0.415	Published SMR 1.32 (0.57, 2.6)	ICD-9 189.0−189.2. Unpublished RR, adjusted for age and sex (see text).
Raaschou-Nielsen et al., 2003	1.20	0.94	1.50	SIR	0.182	0.199	1.20 (0.98, 1.46) for ICD-7 180	RCC.
Radican et al., 2008	1.18	0.47	2.94	Mortality HR	0.166	0.468	None	ICD-8, -9 189.0, ICD-10 C64. Time variable = age; covariates = sex and race. Referent group is workers with no chemical exposures.
Zhao et al., 2005	1.7	0.38	7.9	Mortality RR	0.542	0.775	Incidence RR: 2.0 (0.47, 8.2) Mortality RR no lag: 0.89 (0.22, 3.6) Incidence RR no lag : 2.1 (0.56, 8.1) Boice (2006) SMR: 2.22 (0.89, 4.57)	ICD-9 189. Males only. Adjusted for age, SES, time since first employment, exposure to other carcinogens. 20-yr lag. Mortality results reflect same number exposed cases (10 with no lag) as do incidence results, so no reason to prefer mortality results, but they are used in primary analysis to avoid appearance of "cherry-picking." Overall RR estimated by combining across exposure groups (see text). Boice (2006) cohort overlaps Zhao cohort; just 7 exposed deaths.

Table C-7. Selected RR estimates for renal cell carcinoma associated with TCE exposure from case-control studies[a]

Study	RR estimate	95% LCL	95% UCL	log RR	SE(log RR)	Alternate RR estimates	Comments
Brüning et al., 2003	2.47	1.36	4.49	0.904	0.305	1.80 (1.01, 3.20) for longest job held in industry with TCE exposure	Self-assessed exposure. Adjusted for age, sex, and smoking.
Charbotel et al., 2006	1.88	0.89	3.98	0.631	0.382	1.64 (0.95, 2.84) for full study	Subgroup with good level of confidence about exp assessment. Matched on sex, age. Adjusted for smoking, body mass index.
Dosemeci et al., 1999	1.30	0.9	1.9	0.262	0.191		Adjusted for age, sex, smoking, hypertension and/or use of diuretics and/or anti-hypertension drugs, body mass index.
Pesch et al., 2000	1.24	--[b]	--[b]	0.215	0.094	1.13 with German JEM	With JTEM (job task exposure matrix). Crude OR calculated from data provided in personal communication (see text).
Siemiatycki 1991	0.8	0.3	2.2	−0.223	0.524		"Kidney cancer." SE and 95% CI calculated from reported 90% CIs. Males only; adjusted for age, income, and cigarette smoking index.

[a]The RR estimates are all ORs for incident cases.
[b]Not calculated.

For Boice et al. (1999), only results for "potential routine exposure" were reported for kidney cancer. This is our preferred TCE exposure definition for the Boice study, because it was considered to have less exposure misclassification than "any potential exposure;" however, since the results for the latter definition were not presented, they could not be used in a sensitivity analysis, as was done for lymphoma. Boice et al. (1999) report in general that the SMRs for workers with any potential exposure "were similar to those for workers with daily potential exposure." In their published paper, Morgan et al. (1998) present only SMRs for overall TCE exposure, although the results from internal analyses are presented for exposure subgroups. RR estimates for overall TCE exposure from the internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (Environmental Health Strategies, 1997); from these, the RR estimate from the Cox model which included age and sex was selected, because those are the variables deemed to be important in the published paper. The internal analysis RR estimate was preferred for the primary analysis, and the published SMR result was used in a sensitivity analysis. Raaschou-Nielsen et al. (2003) reported results for RCC and renal pelvis/ureter separately. As discussed above, RCC estimates were used in the primary analysis, and the results for both kidney cancer categories were combined (across sexes as well), assuming a Poisson distribution, and used in a sensitivity analysis. For Radican et al. (2008), the Cox model hazard ratio (HR) from the 2000 follow-up was used. In the Radican et al. (2008) Cox regressions, age was the time variable, and sex and race were covariates. It should also be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE.

For Zhao et al. (2005), no results for an overall TCE effect are reported. We were unable to obtain any overall estimates from the study authors, so, as a best estimate, as was done for lymphoma, the results across the "medium" and "high" exposure groups were combined, under assumptions of group independence, even though the exposure groups are not independent (the "low" exposure group was the referent group in both cases). Unlike for lymphoma, adjustment for exposure to other carcinogens made a considerable difference, so Zhao et al. (2005) also present kidney results with this additional adjustment, with and without a 20-year lag. Estimates of RR with this additional adjustment were selected over those without. In addition, a 20-year lag seemed reasonable for kidney cancer, so the lagged estimates were preferred to the unlagged; unlagged estimates were used in sensitivity analyses. Zhao et al. (2005) present RR estimates for both incidence and mortality. Unlike for lymphoma, the number of exposed incident cases (10 with no lag) was identical to the number of deaths, so there was no reason to prefer the mortality results over the incidence results. (In fact, there were more exposed incident cases [10 vs. 7] after lagging.) However, the mortality results, which yield a lower RR estimate, were selected for the primary analysis to avoid any appearance of “cherry-picking,” and incidence RR estimates were used in sensitivity analyses. A sensitivity analysis was also done using results from Boice et al. (2006) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies and should not be included in the meta-analysis concurrently. Boice et al. (2006) report results for an overall TCE effect for kidney cancer; however, the results are SMR estimates rather than internal comparisons and are based on fewer exposed deaths (7), so either Zhao et al. (2005) estimate is preferred over the Boice et al. (2006) estimate.

Regarding the case-control studies, for Brüning et al. (2003), the results based on self-assessed exposure were preferred because, although TCE exposure was probably under ascertained with this measure, there were greater concerns about the result based on the alternate measure reported—longest-held job in an industry with TCE exposure. Even though this study was conducted in the Arnsberg region of Germany, an area with high prevalence of exposure to TCE, the exposure prevalence in both cases (87%) and controls (79%) seemed inordinately high, and this for not just any job in an industry with TCE exposure, but for the longest-held job. Furthermore, Table V of Brüning et al., which presents this result, states that the result is for longest-held job in industries with TCE *or tetrachloroethylene* exposure. Additionally, some of the industries with exposure to TCE presented in Table V have many jobs that would not entail TCE exposure (e.g., white-collar workers), so the assessment based on industry alone likely has substantial misclassification. Both of these—inclusion of tetrachloroethylene and exposure assessment by industry—could result in overstating TCE exposure prevalence. Results based on the longest-held-job measure were used in a sensitivity analysis.

For Charbotel et al. (2006), results from the analysis that considered “only job periods with a good level of confidence for TCE exposure assessment” (Table 7 of Charbotel et al., 2006) were preferred, as these estimates would presumably be less influenced by exposure misclassification. Estimates from the full study analysis were used in a sensitivity analysis. For Pesch et al. (2000), TCE results were presented for 2 different exposure assessments. Estimates using the job-task-exposure-matrix (JTEM) approach were preferred because they seemed to represent a more comprehensive exposure assessment (see Appendix B, Section II-4); estimates based on the JEM approach were used in a sensitivity analysis. Furthermore, results were presented only by exposure category, with no overall RR estimate reported. Case and control numbers for the different exposure categories were kindly provided by Dr. Pesch (personal communication from Baete Pesch, BGFA, to Cheryl Scott, U.S. EPA, 21 February 2008), and we calculated crude overall ORs for males and females combined for each exposure assessment approach.

C.3.1.2. *Results of Meta-Analyses*

Results from some of the meta-analyses that were conducted on the epidemiological studies of TCE and kidney cancer are summarized in Table C-8. The pooled estimate from the primary random effects meta-analysis of the 14 studies was 1.25 (95% CI: 1.11, 1.41) (see Figure C-5). As shown in Figure C-5, the analysis was dominated by 2 (contributing almost 70% of the weight) or 3 (almost 80% of the weight) large studies. No single study was overly influential; removal of individual studies resulted in RRp estimates that were all statistically significant (all with $p < 0.005$) and that ranged from 1.22 (with the removal of Brüning) to 1.27 (with the removal of Raaschou-Nielsen).

Similarly, the RRp estimate was not highly sensitive to alternate RR estimate selections. Use of the 10 alternate selections, individually, resulted in RRp estimates that were all statistically significant (all with $p < 0.002$) and that ranged from 1.19 to 1.27 (see Table C-8). In fact, as can be seen in Table C-8, all but one of the alternates had negligible impact. The Zhao, Axelson, Brüning, and Charbotel original values and alternate selections were associated with very little weight and, thus, have little influence in the RRp. The Raaschou-Nielsen value carried more weight, but the alternate RR estimate was identical to the original, although with a narrower CI, and so did not alter the RRp. Only the Pesch alternate (with the JEM exposure assessment approach instead of the JTEM approach) had much impact, resulting in an RRp estimate of 1.19 (95% CI: 1.07, 1.32). As noted above, the JTEM approach is preferred. The JEM approach takes jobs into account but not tasks; thus, it is expected to have greater potential for exposure misclassification. Indeed, a comparison of exposure prevalences for the two approaches suggests that the JEM approach is less discriminating about exposure; 42% of cases were defined as TCE-exposed under the JEM approach, but only 18% of cases were exposed under the JTEM approach.

Table C-8. Summary of some meta-analysis results for TCE (overall) and kidney cancer

Analysis	# of studies	Model	Combined RR estimate	95% LCL	95% UCL	Heterogeneity	Comments
All studies	14	Random	1.25	1.11	1.41	None obs	Statistical significance not dependent on single study. No apparent publication bias.
		Fixed	1.25	1.11	1.41		
Cohort	9	Random	1.16	0.96	1.40	None obs	Not significant difference between CC and cohort studies (*p* = 0.23).
		Fixed	1.16	0.96	1.40		Not significant difference between CC and cohort studies (*p* = 0.29).
Case-control	5	Random	1.41	1.08	1.83	Not significant (*p* = 0.17)	
		Fixed	1.32	1.13	1.54		
Alternate RR selections[a]	14	Random	1.25	1.11	1.40−1.41	None obs	With 3 different alternates from Zhao (see Table C-6).
	14	Random	1.27	1.13	1.43	None obs	With Boice (2006) study rather than Zhao
	14	Random	1.25	1.11	1.41	None obs	With estimated female contribution to Axelson.
	14	Random	1.26	1.11	1.41	None obs	With Morgan published SMR.
	14	Random	1.25	1.11	1.40	None obs	With Raaschou-Nielsen all kidney cancer.
	14	Random	1.24	1.10	1.39	None obs	With Brüning longest job held in industry with TCE.
	14	Random	1.25	1.11	1.41	None obs	With Charbotel full study
	14	Random	1.19	1.07	1.32	None obs	With Pesch JEM.
Highest exposure groups	9	Random	1.59	1.26	2.01	None obs	
	12	Random	1.53	1.23	1.91	None obs	Using RR = 1 for Anttila, Axelson, and Hansen (see text). See Table C-10 for alternate RR selection results.

[a]Changing the primary analysis by one alternate RR each time.

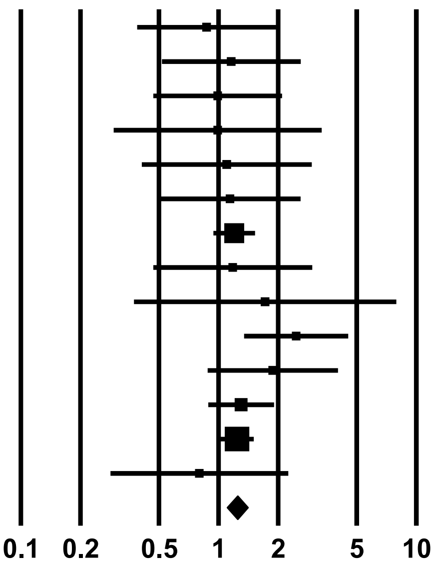
obs = observable.

TCE and Kidney Cancer

Study name	Risk ratio	Lower limit	Upper limit	p-Value
Anttila 1995	0.870	0.391	1.937	0.7330
Axelson 1994	1.160	0.521	2.582	0.7162
Boice 1999	0.990	0.472	2.077	0.9788
Greenland 1994	0.990	0.298	3.293	0.9869
Hansen 2001	1.100	0.413	2.931	0.8488
Morgan 1998 unpub RR	1.143	0.507	2.576	0.7472
Raaschou-Nielsen 2003 RCC	1.200	0.950	1.516	0.1262
Radican 2008	1.180	0.472	2.951	0.7234
Zhao 2005 mort 20 y lag	1.720	0.377	7.853	0.4840
Bruning 2003	2.470	1.359	4.488	0.0030
Charbotel 2007- high conf re:exp	1.880	0.889	3.976	0.0985
Dosemeci 1999	1.300	0.895	1.889	0.1687
Pesch 2000 JTEM	1.240	1.030	1.492	0.0227
Siemiatycki 1991	0.800	0.287	2.233	0.6700
	1.251	1.110	1.410	0.0002

Statistics for each study

Risk ratio and 95% CI



random effects model; same for fixed

Figure C-5. Meta-analysis of kidney cancer and overall TCE exposure. The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the pooled RR estimate and the horizontal extremes depict the 95% CI limits.

There was no apparent heterogeneity across the 14 studies, i.e., the random effects model and the fixed effect model gave the same results. Nonetheless, subgroup analyses were done examining the cohort and case-control studies separately. With the random effects model (and tau-squared not pooled across subgroups), the resulting RRp estimates were 1.16 (95% CI: 0.96, 1.40) for the cohort studies and 1.41 (1.08, 1.83) for the case-control studies. There was heterogeneity in the case-control subgroup, but it was not statistically significant and the I^2 value of 38% suggests that the extent of the heterogeneity in this subgroup was low-to-moderate. Nor was the difference between the RRp estimates for the cohort and case-control subgroups statistically significant under either the random effects model or the fixed effect model. Further quantitative investigations of heterogeneity were not pursued because of database limitations and, in any event, there is no evidence for heterogeneity of study results in this database. A qualitative discussion of some potential sources of heterogeneity across studies is nonetheless included in Section C.3.3.

As discussed in Section C.1, publication bias was examined in several different ways. The funnel plot in Figure C-6 shows little relationship between RR estimate and study size, and, indeed, none of the other tests performed found any evidence of publication bias. Duval and Tweedie's trim-and-fill procedure, for example, determined that no studies were missing from the funnel plot, i.e., there was no asymmetry to counterbalance. Similarly, the results of a cumulative meta-analysis, incorporating studies with increasing SE one at a time, shows no evidence of a trend of increasing effect size with addition of the less precise studies. Including the 3 most precise studies, reflecting 78% of the weight, the RRp goes from 1.24 to 1.22 to 1.23. The addition of the Brüning study brings the RRp to 1.32 and the weight to 82%. After the addition of the next 5 studies, the RRp stabilizes at about 1.26, and further addition of the 5 least precise studies has little impact.

C.3.2. Kidney Cancer Effect in the Highest Exposure Groups

C.3.2.1. *Selection of RR Estimates*

The selected RR estimates for kidney cancer in the highest TCE exposure categories, for studies that provided such estimates, are presented in Table C-9. Five of the 9 cohort studies and 4 of the 5 case-control studies reported kidney cancer risk estimates categorized by exposure level. As in Section C.3.1.1 for the overall risk estimates, estimates for RCC were preferentially selected when presented, and, wherever possible, RR estimates for males and females combined were used.

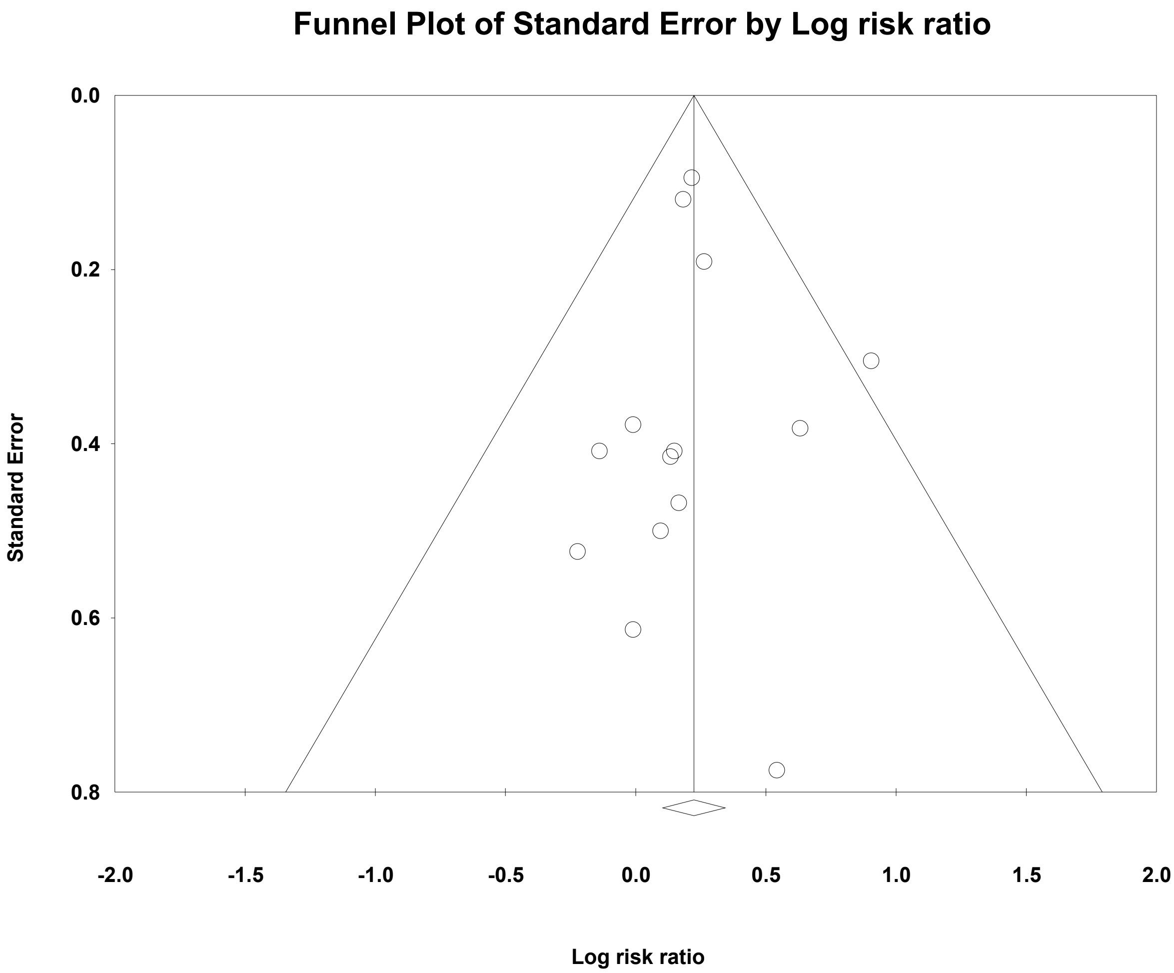


Figure C-6. Funnel plot of SE by log RR estimate for TCE and kidney cancer studies

Table C-9. Selected RR estimates for kidney cancer risk in highest TCE exposure groups

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE(log RR)	Alternate RR estimates	Comments
Anttila et al., 1995				100+ μmol/L U-TCA [a]			1.0 assumed	Reported high exposure group results for some cancer sites but not kidney.
Axelson et al., 1994				≥2 yr exposure and 100+ mg/L U-TCA			1.0 assumed	Reported high exposure group results for some cancer sites but not kidney.
Boice et al., 1999	0.69	0.22	2.12	≥5 yr exp	−0.371	0.578	None	Mortality RR. ICD-9 189.0−189.2. For potential routine or intermittent exposure. adjusted for date of birth, dates 1st and last employed, race, and sex. Referent group is workers not exposed to any solvent.
Hansen et al., 2001				≥1080 mos × mg/m^3			1.0 assumed	Reported high exposure group results for some cancer sites but not kidney.
Morgan et al., 1998	1.59	0.68	3.71	High cumulative exposure score	0.464	0.433	1.89 (0.85, 4.23) for med/high peak vs. low/no	Mortality RR. ICD-9 189.0−189.2. Adjusted for age and sex.
Raaschou-Nielsen et al., 2003	1.7	1.1	2.4	≥5 yrs in subcohort with expected higher exposure levels	0.531	0.183	1.4 (0.99, 1.9) ICD-7 180 ≥5 yrs in total cohort	SIR. RCC.
Radican et al., 2008	1.11	0.35	3.49	>25 unit-yr	0.104	0.582	Blair et al. (1998) incidence RR 0.9 (0.3, 3.2)	Mortality HR. ICD-8, -9 189.0, ICD-10 C64. Male and female results presented separately and combined (see text). Referent group is workers with no chemical exposures.

Table C-9. Selected RR estimates for kidney cancer risk in highest TCE exposure groups (continued)

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE(log RR)	Alternate RR estimates	Comments
Zhao et al., 2005	7.40	0.47	116	High exposure score	2.00	1.41	Mortality RR: 1.82 (0.09, 38.6) Incidence RR no lag: 7.71 (0.65, 91.4) Mortality RR no lag: 0.96 (0.09, 9.91) Boice 2006 mortality RR: 2.12 (0.63, 7.11) for ≥5 yrs as test stand mechanic; 3.13 (0.74,13.2) for ≥4 test-yr engine flush	Incidence RR. ICD-9 189. Males only. Adjusted for age, SES, time since first employment, exposure to other carcinogens. 20-yr lag. Incidence results reflect more exposed cases (4 with no lag) than do mortality results (3), so they are used in primary analysis.
Brüning et al., 2003	2.69	0.84	8.66	≥20 yrs self-assessed exposure	0.990	0.595	None	Incidence OR. RCC. Adjusted for age, sex, and smoking.
Charbotel et al., 2006	3.34	1.27	8.74	High cumulative dose	1.21	0.492	3.80 (1.27, 11.40) for high cum + peaks 1.96 (0.71, 5.37) for high cum + peaks in full study 2.63 (0.79, 8.83) for high cum in full study	Incidence OR. RCC. In subgroup with good level of confidence for TCE exposure. Adjusted for smoking and body mass index. Matched on sex and age. Alternate full study estimates were additionally adjusted for exposure to cutting fluids and other petroleum oils.

Table C-9. Selected RR estimates for kidney cancer risk in highest TCE exposure groups (continued)

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE(log RR)	Alternate RR estimates	Comments
Pesch et al., 2000	1.4	0.9	2.1	Substantial	0.336	0.219	1.2 (0.9, 1.7) for JEM	Incidence OR. RCC. JTEM approach. Adjusted for age, study center, and smoking. Sexes combined.
Siemiatycki 1991	0.8	0.2	3.4	Substantial	−0.233	0.736	none	Incidence OR. Kidney cancer. SE and 95% CI calculated from reported 90% CIs. Males only; adjusted for age, income, and cigarette smoking index.

[a]Mean personal trichloroacetic acid in urine. 1 μmol/L = 0.1634 mg/L.

Three of the 9 cohort studies (Anttila et al., 1995; Axelson et al., 1994; Hansen et al., 2001) did not report kidney cancer risk estimates categorized by exposure level even though these same studies reported such estimates for selected other cancer sites. To address this reporting bias, attempts were made to obtain the results from the primary investigators, and, failing that, an alternate analysis was performed in which null estimates (RR = 1.0) were included for all 3 studies. This alternate analysis was then used as the main analysis, e.g., the basis of comparison for the sensitivity analyses. For the SE (of the logRR) estimates for these null estimates, SE estimates from other sites for which highest-exposure-group results were available were used. For Anttila et al. (1995), the SE estimate for liver cancer in the highest exposure group was used, because liver cancer and kidney cancer had similar numbers of cases in the overall study (5 and 6, respectively). For Axelson et al. (1994), the SE estimate for NHL in the highest exposure group was used, because NHL and kidney cancer had similar numbers of cases in the overall study (5 and 6, respectively). For Hansen et al. (2001), the SE estimate for NHL in the highest exposure group was used, because NHL was the only cancer site of interest in this assessment for which highest-exposure-group results were available.

For Boice et al. (1999), only results for workers with "any potential exposure" (rather than "potential routine exposure") were presented by exposure category, and the referent group is workers not exposed to any solvent. For Morgan et al. (1998), the primary analysis used results for the cumulative exposure metric, and a sensitivity analysis was done with the results for the peak exposure metric.

For Radican et al. (2008), it should be noted that the referent group is workers with no chemical exposures, not just no TCE exposure. In addition, exposure group results were reported separately for males and females and were combined for this assessment using inverse-variance weighting, as in a fixed effect meta-analysis. Radican et al. (2008) present only mortality HR estimates by exposure group; however, in an earlier follow-up of this same cohort, Blair et al. (1998) present both incidence and mortality RR estimates by exposure group. The mortality RR estimate based on the more recent follow-up of Radican et al. (2008) (6 deaths in the highest exposure group) was used in the primary analysis, while the incidence RR estimate based on similarly combined results from Blair et al. (1998) (4 cases) was used as an alternate estimate in a sensitivity analysis.

Zhao et al. (2005) present kidney cancer RR estimates adjusted for exposure to other carcinogens, because, unlike for lymphoma, this adjustment made a considerable difference. Estimates of RR with this additional adjustment were selected over those without. Furthermore, the kidney results were presented with and without a 20-year lag. A 20-year lag seemed reasonable for kidney cancer, so the lagged estimates were preferred to the unlagged; unlagged estimates were used in sensitivity analyses. In addition, the incidence results reflect more cases (4 with no lag) in the highest exposure group than do the mortality results (3), so the incidence result (with the 20-year lag) was used for the primary analysis, and the unlagged incidence result and the mortality results were used in a sensitivity analysis. Sensitivity analyses were also done using results from Boice et al. (2006) in place of the Zhao et al. (2005) RR estimate. The cohorts for these studies overlap, so they are not independent studies. Boice et al. (2006) report mortality RR estimates for kidney cancer by years worked as a test stand mechanic, a job with potential TCE exposure, and by a measure that weighted years with potential exposure from engine flushing by the number of flushes each year. No results were presented for a third metric, years worked with potential exposure to any TCE, because the Cox proportional hazards model did not converge. The Boice et al. (2006) estimates are adjusted for years of birth and hire and for hydrazine exposure.

For Charbotel et al. (2006), results from the analysis that considered "only job periods with a good level of confidence for TCE exposure assessment" (Table 7 of Charbotel et al., 2006) were preferred, as these estimates would presumably be less influenced by exposure misclassification. Estimates from the full study analysis, additionally adjusted for exposure to cutting fluids and other petroleum oils, were used in a sensitivity analysis. Additionally, the high cumulative dose results were preferred, but the results for high cumulative dose + peaks were included in sensitivity analyses. For Pesch et al. (2000), TCE results were presented for two different exposure assessments. As discussed above, estimates using the JTEM approach were preferred because they seemed to represent a more comprehensive exposure assessment; estimates based on the JEM approach were used in a sensitivity analysis.

C.3.2.2. *Results of Meta-Analyses*

Results from the meta-analyses that were conducted for kidney cancer in the highest exposure groups are summarized at the bottom of Table C-8 and reported in more detail in Table C-10. The pooled RR estimate from the random effects meta-analysis of the 9 studies with results presented for exposure groups was 1.59 (95% CI: 1.26, 2.01) (see Figure C-7). The RRp estimate from the primary random effects meta-analysis with null RR estimates (i.e., 1.0) included for Anttila, Axelson, and Hansen to address reporting bias (see above) was 1.53 (1.23, 1.91) (see Figure C-8). The inclusion of these 3 additional studies contributed just under 8% of the total weight. As with the overall kidney cancer meta-analyses, the meta-analyses of the highest-exposure groups were dominated by 2 studies (Raaschou-Nielsen and Pesch), which provided about 66% of the weight. No single study was overly influential; removal of individual studies resulted in RRp estimates that were all statistically significant (all with $p < 0.02$) and that ranged from 1.43 (with the removal of Raaschou-Nielsen) to 1.58 (with the removal of Boice [1999] or Pesch).

Similarly, the RRp estimate was not highly sensitive to alternate RR estimate selections. Use of the 12 alternate selections, individually, resulted in RRp estimates that were all statistically significant (all with $p < 0.002$) and that ranged from 1.42 to 1.55, with all but 2 of the alternate selections yielding RRp estimates in the narrow range of 1.49−1.55 (see Table C-10). The lowest RRp estimates, 1.42 in both cases, were obtained when the alternate selections involved the 2 large studies. One of the alternate selections was for Raaschou-Nielsen, with a highest-exposure group estimate for all kidney cancer in the total cohort, rather than RCC in the subcohort expected to have higher exposure levels. The latter value is strongly preferred because, as discussed above, the subcohort is likely to have less exposure misclassification. Furthermore, RCC is very different from other types of kidney cancer, and TCE, if an etiological factor, may not be etiologically associated with all kidney cancers, so using the broad category may dilute a true association with RCC, if one exists. The other alternate selection with a considerable impact on the RRp estimate was for Pesch, with the highest exposure group result based on the JEM exposure assessment approach, rather than the JTEM approach. As discussed above, the JTEM approach is preferred because it seemed to be a more comprehensive and discriminating approach, taking actual job tasks into account, rather than just larger job categories. Thus, although results with these alternate selections are presented for comprehensiveness and transparency, the primary analysis is believed to reflect better the potential association between kidney cancer (in particular, RCC) and TCE exposure.

There was no observable heterogeneity across the studies for any of the meta-analyses conducted with the highest-exposure groups, including those in which RR values for Anttila, Axelson, and Hansen were assumed. No subgroup analyses (e.g., cohort vs. case-control studies) were done with the highest exposure group results.

Table C-10. Summary of some meta-analysis results for TCE (highest exposure groups) and kidney cancer

Analysis	Model	Combined RR estimate	95% LCL	95% UCL	Heterogeneity	Comments
Analysis based on reported results	Random	1.59	1.26	2.01	None obs (fixed = random)	
Primary analysis	Random	**1.53**	**1.23**	**1.91**	None obs	Includes assumed values for Anttila, Axelson, and Hansen (see text). Statistical significance not dependent on single study.
Alternate RR selections[a]	Random	1.52	1.22	1.90	None obs	With Blair et al. (1998) incidence RR instead of Radican mortality HR.
	Random	1.55	1.24	1.94	None obs	With Morgan peak metric.
	Random	1.42	1.15	1.75	None obs	With Raaschou-Nielsen for all kidney cancer ≥5 yrs in total cohort.
	Random	1.51−1.54	1.21−1.23	1.89−1.92	None obs	With Zhao incidence unlagged and mortality with and without lag.
	Random	1.53−1.54	1.23−1.24	1.91−1.92	None obs	With Boice (2006) alternates for Zhao (see text).
	Random	1.49−1.52	1.19−1.22	1.86−1.91	None obs	With Charbotel high cumulative dose + peaks in subgroup; and high cumulative dose and high cumulative dose + peaks in full study additionally adjusted for exposure to cutting fluids and other petroleum oils..
	Random	1.42	1.16	1.74	None obs	With Pesch JEM.

[a]Changing the primary analysis by one alternate RR each time.

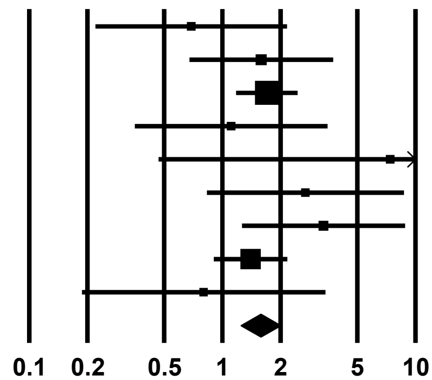
obs = observable.

TCE and Kidney Cancer - highest exposure groups

Study name	Risk ratio	Lower limit	Upper limit	p-Value
Boice 1999	0.690	0.222	2.142	0.5208
Morgan 1998	1.590	0.681	3.714	0.2840
Raaschou-Nielsen 2003	1.700	1.189	2.431	0.0037
Radican 2008	1.110	0.355	3.470	0.8576
Zhao 2005 inc 20y lag	7.400	0.471	116.249	0.1544
Bruning 2003	2.690	0.838	8.634	0.0963
Charbotel 2007 good conf re:exp	3.340	1.273	8.761	0.0142
Pesch 2000 - JTEM	1.400	0.911	2.151	0.1244
Siemiatycki 1991	0.800	0.189	3.385	0.7618
	1.586	1.255	2.006	0.0001

Statistics for each study

Risk ratio and 95% CI



random effects model

Figure C-7. Meta-analysis of kidney cancer and TCE exposure—highest exposure groups. The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the pooled RR estimate and the horizontal extremes depict the 95% CI limits.

TCE and Kidney Cancer - highest exposure groups

Study name	Risk ratio	Lower limit	Upper limit	p-Value
Boice 1999	0.690	0.222	2.142	0.5208
Morgan 1998	1.590	0.681	3.714	0.2840
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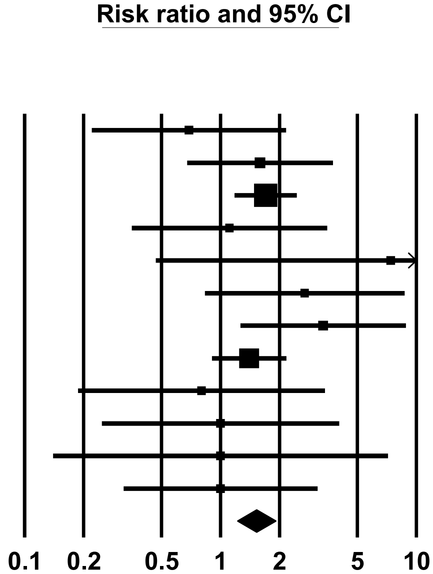
Charbotel 2007 good conf re:exp	3.340	1.273	8.761	0.0142
Pesch 2000 - JTEM	1.400	0.911	2.151	0.1244
Siemiatycki 1991	0.800	0.189	3.385	0.7618
Antilla	1.000	0.250	3.998	1.0000
Axelson	1.000	0.141	7.099	1.0000
Hansen	1.000	0.323	3.098	1.0000
	1.531	1.225	1.913	0.0002



random effects model; same for fixed

Figure C-8. Meta-analysis of kidney cancer and TCE exposure—highest exposure groups, with assumed null RR estimates for Anttila, Axelson, and Hansen (see text).

C.3.3. Discussion of Kidney Cancer Meta-Analysis Results

For the most part, the meta-analyses of the overall effect of TCE exposure on kidney cancer suggest a small, statistically significant increase in risk. The pooled estimate from the primary random effects meta-analysis of the 14 studies was 1.25 (95% CI: 1.11, 1.41). Although the analysis was dominated by 2−3 large studies that contribute 70−80% of the weight, the pooled estimate was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. The largest downward impacts were from the removal of the Brüning study, resulting in an RRp estimate of 1.22 (95% CI: 1.08, 1.37), and from the substitution of the Pesch JTEM RR estimate with the RR estimate based on the JEM approach, resulting in an RRp estimate of 1.19 (1.07, 1.32). Thus, the finding of an increased risk of kidney cancer associated with TCE exposure is robust. Furthermore, there is no evidence of publication bias in this data set.

In addition, there was no heterogeneity observed across the results of the 14 studies. When subgroup analyses were done of cohort and case-control studies separately, there was some observable heterogeneity among the case-control studies, but it was not statistically significant ($p = 0.17$) and the I^2 value of 38% suggested the extent of the heterogeneity was low-to-moderate. The increased risk of kidney cancer was strengthened in the case-control study analysis and weakened in the cohort study analysis, but the difference between the 2 RRp estimates was not statistically significant. One difference between the case-control and cohort studies is that the case-control studies were of RCC and almost all of the cohort studies were of all kidney cancers, including renal pelvis. As discussed above, RCC is very different from other types of kidney cancer, and TCE, if an etiological factor, may not be etiologically associated with all kidney cancers, so using the broad category may dilute a true association with RCC, if one exists.

With respect to the nonsignificant heterogeneity in the 5 case-control studies, these studies differ in TCE exposure potential to the underlying population from which case and control subjects were identified, and this may be a source of some heterogeneity. Prevalence of exposure to TCE among cases in these studies was 27% in Charbotel et al. (2006) (for high-level-of-confidence jobs), 18% in Brüning et al. (2003) (for self-assessed exposure), 18% in Pesch et al. (2000), 13% in Dosemeci et al. (1999) and 1% in Siemiatycki (1991). Both Brüning et al. (2003) and Charbotel et al. (2006) are studies designed specifically to assess RCC and TCE exposure. These studies were carried out in geographical areas with both a high prevalence and a high degree of TCE exposure. Some information is provided in these and accompanying papers to describe the nature of exposure, making it possible to estimate the order of magnitude of exposure, even though there were no direct measurements (Cherrie et al., 2001; Brüning et al., 2003; Fevotte et al., 2006). The Charbotel et al. (2006) study was carried out in the Arve Valley region in France, where TCE exposure was through metal-degreasing activity in small shops involved in the manufacturing of screws and precision metal parts (Fevotte et al., 2006). Industrial hygiene data from shops in this area indicated high intensity TCE exposures of 100 ppm or higher, particularly from exposures from hot degreasing processes. Considering exposure only from the jobs with a high level of confidence about exposure, 18% of exposed cases were identified with high cumulative exposure to TCE. The source population in the Brüning et al. (2003) study includes the Arnsberg region in Germany, which also has a high prevalence of TCE exposure. A large number of small companies used TCE in metal degreasing in small workrooms. Subjects in this study also described neurological symptoms previously associated with higher TCE intensities. While subjects in the Brüning et al. (2003) study had potential high TCE exposure intensity, average TCE exposure in this study is considered lower than that in the Charbotel et al. (2006) study because the base population was enlarged beyond the Arnsberg region to areas which did not have the same focus of industry.

Siemiatycki (1991), Dosemeci et al. (1999), and Pesch et al. (2000) are population-based studies. Pesch et al. (2000) includes the Arnsberg area and 4 other regions. Sources of exposure to TCE and other chlorinated solvents are much less well defined, and most subjects identified with TCE exposure probably had minimal contact; estimated average concentrations to exposed subjects were of about 10 ppm or less (NRC, 2006). Neither Dosemeci et al. (1999) nor Siemiatycki (1991) describe the nature of the TCE exposure. TCE exposure potential in these studies is likely lower than in the three other studies and closer to background. Furthermore, the use of generic job-exposure-matrices for exposure assessment in these studies may result in a greater potential for exposure misclassification bias.

Nine of the 14 studies categorized results by exposure level. Three other studies reported results for other cancer sites by exposure level, but not kidney cancer; thus, to address this reporting bias, null values (i.e., RR estimates of 1.0) were used for these studies. Different exposure metrics were used in the various studies, and the purpose of combining results across the different highest exposure groups was not to estimate an RRp associated with some level of exposure, but rather to see the impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of kidney cancer, the effects should be more apparent in the highest exposure groups. Indeed, the RRp estimate from the primary meta-analysis of the highest exposure group results was 1.53 (95% CI: 1.23, 1.91), which is greater than the RRp estimate of 1.25 (95% CI: 1.11, 1.41) from the overall exposure analysis. This result for the highest exposure groups was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. Heterogeneity was not observed in any of the analyses. The robustness of this finding lends substantial support to a conclusion that TCE exposure increases the risk of kidney cancer.

C.4. META-ANALYSIS FOR LIVER CANCER

C.4.1. Overall Effect of TCE Exposure

C.4.1.1. *Selection of RR Estimates*

The selected RR estimates for liver cancer associated with TCE exposure from the epidemiological studies are presented in Table C-11. There were no case-control studies for liver cancer and TCE exposure that were selected for inclusion in the meta-analysis (see Appendix B, Section II-9), so all of the relevant studies are cohort studies. All of the studies reported results for liver cancers plus cancers of the gall bladder and extrahepatic biliary passages (i.e., ICD-7 155.0 + 155.2; ICD-8 and -9 155 + 156). Three of the studies also report results for liver cancer alone (ICD-7 155.0; ICD-8 and -9 155). For the primary analysis, results for cancers of the liver, gall bladder, and biliary passages combined were selected, for the sake of consistency, since these were reported in all the studies. An alternate analysis was also done using results for liver cancer alone for the 3 studies that reported them and the combined liver cancer results for the remainder of the studies.

As for lymphoma and kidney cancer, many of the studies provided RR estimates only for males and females combined, and we are not aware of any basis for a sex difference in the effects of TCE on liver cancer risk; thus, wherever possible, RR estimates for males and females combined were used. The only study of much size (in terms of number of liver cancer cases) that provided results separately by sex was Raaschou-Nielsen (2003). The results of this study suggest that liver cancer risk in females might be slightly higher than the risk in males, but the number of female cases is small (primary liver cancer SIR: males 1.1 [95% CI: 0.74, 1.64; 27 cases], females 2.8 [1.13, 5.80; 7 cases]; gallbladder and biliary passage cancers SIR: males 1.1 [0.61, 1.87; 14 cases]; females 2.8 [1.28, 5.34; 9 cases]). Radican et al. (2008) report HRs for liver/biliary passage cancers combined of 1.36 (95% CI: 0.59, 3.11; 28 deaths) for males and 0.74 (95% CI: 0.18, 2.97; 3 deaths) for females, but these results are based on fewer cases, especially in females.

Table C-11. Selected RR estimates for liver cancer associated with TCE exposure (overall effect) from cohort studies

Study	RR	95% LCL	95% UCL	RR type	log RR	SE(log RR)	Alternate RR estimates	Comments
Anttila et al., 1995	1.89	0.86	3.59	SIR	0.637	0.333	2.27 (0.74, 5.29) for 155.0 alone	ICD-7 155.0 + 155.1; combined assuming Poisson distribution.
Axelson et al., 1994	1.41	0.38	3.60	SIR	0.344	0.5	1.34 (0.36, 3.42) with estimated female contribution to SIR added (see text)	ICD-7 155. Results reported for males only, but there was a small female component to the cohort.
Boice et al., 1999	0.54	0.15	1.38	SMR	−0.616	0.5	0.81 (0.45, 1.33) for any potential exposure	ICD-9 155 + 156. For potential routine exposure.
Greenland et al., 1994	0.54	0.11	2.63	OR	−0.616	0.810	None	ICD-8 155 + 156. Nested case-control study.
Hansen et al., 2001	2.1	0.7	5.0	SIR	0.742	0.447	None	ICD-7 155. Male and female results reported separately; combined assuming Poisson distribution.
Morgan et al., 1998	1.48	0.56	3.91	SMR	0.393	0.495	Published SMR 0.98 (0.36, 2.13)	ICD-9 155 + 156. Unpublished RR, adjusted for age and sex (see text).
Raaschou-Nielsen et al., 2003	1.35	1.03	1.77	SIR	0.300	0.138	1.28 (0.89, 1.80) for ICD-7 155.0	ICD-7 155.0 + 155.1. Results for males and females and different liver cancer types reported separately; combined assuming Poisson distribution.
Radican et al., 2008	1.12	0.57	2.19	Mortality HR	0.113	0.343	1.25 (0.31, 4.97) for ICD-8, -9 155.0	ICD-8, -9 155 + 156, ICD-10 C22-C24. Time variable = age; covariates = sex, race. Referent group is workers with no chemical exposures.
Boice et al., 2006	1.28	0.35	3.27	SMR	0.247	0.5	1.0 assumed for Zhao et al. (2005)	ICD-9 155 + 156. Boice et al. (2006) used in lieu of Zhao et al. (2005) because Zhao et al. (2005) do not report liver cancer results. Boice (2006) cohort overlaps Zhao cohort.

Most of the selections in Table C-11 should be self-evident, but some are discussed in more detail here, in the order the studies are presented in the table. For Axelson et al. (1994), in which a small subcohort of females was studied but only results for the larger male subcohort were reported, the reported male-only results were used in the primary analysis; however, as for lymphoma and kidney cancer, an attempt was made to estimate the female contribution to an overall RR estimate for both sexes and its impact on the meta-analysis. Axelson et al. (1994) reported that there were no cases of liver cancer observed in females, but the expected number was not presented. To estimate the expected number, the expected number for males was multiplied by the ratio of female-to-male person-years in the study and by the ratio of female-to-male age-adjusted incidence rates for liver cancer. The male results and the estimated female contribution were then combined into an RR estimate for both sexes assuming a Poisson distribution, and this alternate RR estimate for the Axelson et al. (1994) study was used in a sensitivity analysis.

For Boice et al. (1999), results for "potential routine exposure" were selected for the primary analysis, because this exposure category was considered to have less exposure misclassification, and results for "any potential exposure" were used in a sensitivity analysis. To estimate the SE(logRR) for the alternate RR selection, it was assumed that the number of exposed cases (deaths) was 15. The actual number was not presented, but 15 was the number that allowed us to reproduce the reported CIs. The number suggested by exposure level in Boice et al. (1999) Table 9 is 13; however, it may be that exposure level data were not available for all the cases. In their published paper, Morgan et al. (1998) present only SMRs for overall TCE exposure, although the results from internal analyses are presented for exposure subgroups. RR estimates for overall TCE exposure from the internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (Environmental Health Strategies, 1997); from these, the RR estimate from the Cox model which included age and sex was selected, because those are the variables deemed to be important in the published paper. The internal analysis RR estimate was preferred for the primary analysis, and the published SMR result was used in a sensitivity analysis.

Raaschou-Nielsen et al. (2003) reported results for primary liver cancer (ICD-7 155.0), gallbladder and biliary passage cancers (ICD-7 155.1), and unspecified liver cancers (ICD-7 156) separately. As discussed above, RR estimates for cancers of the liver, gall bladder, and biliary passages combined were preferred for the primary analysis; thus, the results for primary liver cancer and gallbladder/biliary passage cancers were combined (across sexes as well), assuming a Poisson distribution. The results for primary liver cancer only (similarly combined across sexes) were used in an alternate analysis. The results for unspecified liver cancers (ICD-7 156) were not included in any analyses because, under the ICD-7 coding, 156 can include secondary liver cancers. For Radican et al. (2008), the Cox model hazard ratio (HR) from the 2000 follow-up was used. In the Radican et al. (2008) Cox regressions, age was the time variable, and sex and race were covariates. It should also be noted that the referent group is composed of workers with no chemical exposures, not just no exposure to TCE.

Zhao et al. (2005) did not present RR estimates for liver cancer; thus, results from Boice et al. (2006) were used in the primary analysis. The cohorts for these studies overlap, so they are not independent studies. Zhao et al. (2005), however, was our preferred study for lymphoma and kidney cancer results; thus, in a sensitivity analysis, a null value (RR = 1.0) was assumed for Zhao et al. (2005) to address the potential reporting bias. The SE estimate for kidney cancer (incidence with 0 lag) was used as the SE for the liver cancer. (It is not certain that there was a reporting bias in this case. In the "Methods" section of their paper, Zhao et al. [2005] list the cancer sites examined in the cohort, and liver was not listed; it is not clear if the list of sites was determined *a priori* or *post hoc*.) Also, on the issue of potential reporting bias, the Siemiatycki (1991) study should be mentioned. This study was a case-control study for multiple cancer sites, but only the more common sites, in order to have greater statistical power. Thus, NHL and kidney cancer results were available, but not liver cancer results. Because no liver results were presented for any of the chemicals, this is not a case of reporting bias.

C.4.1.2. *Results of Meta-Analyses*

Results from some of the meta-analyses that were conducted on the epidemiological studies of TCE and liver cancer are summarized in Table C-12. The pooled estimate from the primary random effects meta-analysis of the 9 studies was 1.33 (95% CI: 1.09, 1.64) (see Figure C-9). As shown in Figure C-9, the analysis was dominated by one large study (contributing about 57% of the weight). That large study was critical in terms of statistical significance of the RRp estimate. Without the large Raaschou-Nielsen study, the RRp estimate does not change noticeably, but it is no longer statistically significant (RRp = 1.31; 95% CI: 0.96, 1.79). No other single study was overly influential; removal of any of the other individual studies resulted in RRp estimates that were all statistically significant and that ranged from 1.29 (with the removal of Anttila) to 1.39 (with the removal of Boice [1999]).

Table C-12. Summary of some meta-analysis results for TCE and liver cancer

Analysis	# of studies	Model	Combined RR estimate	95% LCL	95% UCL	Heterogeneity	Comments
All studies (all cohort studies)	9	Random	1.33	1.09	1.64	None obs (fixed = random)	Statistical significance not dependent on single study, except for Raaschou-Nielsen, without which *p* = 0.08. No apparent publication bias.
		Fixed	1.33	1.09	1.64		
All studies; liver cancer only, when available	9	Random	1.31	1.02	1.67	None obs	Used RR estimates for liver cancer alone for the 3 studies that presented these; remaining RR estimates are for liver and gall bladder/biliary passage cancers.
Alternate RR selections[a]	9	Random	1.33	1.08	1.63	None obs	With 1.0 assumed for Zhao in lieu of Boice (2006) (see text).
	9	Random	1.29	1.06	1.56	None obs	With Boice (1999) any potential exposure rather than potential routine exposure.
	9	Random	1.33	1.09	1.63	None obs	With estimated female contribution to Axelson.
	9	Random	1.30	1.07	1.59	None obs	With Morgan published SMR.
Highest exposure groups	6	Random	1.32	0.93	1.86	None obs	
	8	Random	1.28	0.93	1.77	None obs	Primary analysis. Using RR = 1 for Hansen and Zhao (see text).
	7−8	Random	1.24−1.26	0.88−0.91	1.73−1.82	None obs	Using alternate selections for Morgan and Raaschou-Nielsen and excluding Axelson.[a]

[a]Changing the primary analysis by one alternate RR each time.

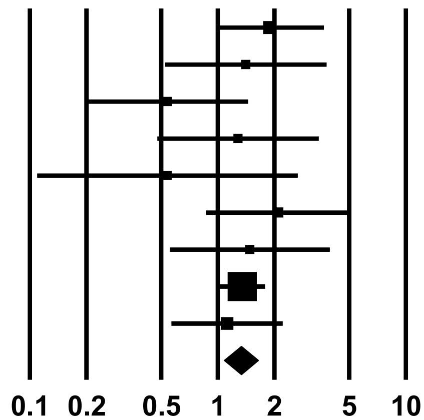
obs = observable.

TCE and Liver Cancer

Study name	Risk ratio	Lower limit	Upper limit	p-Value
Anttila 1995	1.890	0.983	3.632	0.056
Axelson 1994	1.410	0.529	3.757	0.492
Boice 1999	0.540	0.203	1.439	0.218
Boice 2006	1.280	0.480	3.410	0.622
Greenland 1994	0.540	0.110	2.640	0.447
Hansen 2001	2.100	0.874	5.045	0.097
Morgan 1998 unpub RR	1.481	0.561	3.909	0.428
Raaschou-Nielsen 2003	1.350	1.030	1.770	0.030
Radican 2008	1.120	0.571	2.195	0.741
	1.334	1.088	1.636	0.006

(Statistics for each study)

Risk ratio and 95% CI



random effects model; same for fixed

Figure C-9. Meta-analysis of liver cancer and TCE exposure. The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the pooled RR estimate and the horizontal extremes depict the 95% CI limits.

As discussed in Section C.4.1.1, all of the 9 studies presented results for liver and gall bladder/biliary passage cancers combined, and these results were the basis for the primary analysis discussed above. An alternate analysis was performed substituting, simultaneously, results for liver cancer alone for the 3 studies for which these were available. The RRp estimate from this analysis was slightly lower than the one based entirely on results from the combined cancer categories (1.31; 95% CI: 1.02, 1.67). This result was driven by the fact that the RR estimate from the large Raaschou-Nielsen et al. (2003) study decreased from 1.35 for liver and gall bladder/biliary passage cancers combined to 1.28 for liver cancer alone.

Similarly, the RRp estimate was not highly sensitive to other alternate RR estimate selections. Use of the 4 other alternate selections, individually, resulted in RRp estimates that were all statistically significant (all with $p < 0.02$) and that ranged from 1.29 to 1.33 (see

Table C-12). In fact, as can be seen in Table C-12, only one of the alternates had notable impact. The Boice (2006), Zhao, and Axelson original values and alternate selections were associated with very little weight and, thus, have little influence in the RRp. Using the Boice (1999) alternate RR estimate based on any potential exposure rather than potential routine exposure decreased the RRp slightly from 1.33 to 1.29. The alternate Boice (1999) RR estimate is actually larger than the original value (0.81 vs. 0.54); however, use of the less discriminating exposure metric captures more liver cancer deaths, causing the weight of that study to increase from about 4.3% to almost 15%.

There was no apparent heterogeneity across the nine studies, i.e., the random effects model and the fixed effect model gave the same results. Furthermore, all of the liver cancer studies were cohort studies, so no subgroup analyses examining cohort and case-control studies separately, as was done for lymphoma and kidney cancer, were conducted. No alternate quantitative investigations of heterogeneity were pursued because of database limitations and, in any event, there is no evidence for heterogeneity of study results in this database.

As discussed in Section C.1, publication bias was examined in several different ways. The funnel plot in Figure C-10 shows little relationship between RR estimate and study size, and, indeed, none of the other tests performed found any evidence of publication bias. Duval and Tweedie's trim-and-fill procedure, for example, suggested that no studies were missing from the funnel plot, i.e., there was no asymmetry to counterbalance. Similarly, the results of a cumulative meta-analysis, incorporating studies with increasing SE one at a time, shows no evidence of a trend of increasing effect size with addition of the less precise studies. The Raaschou-Nielsen study contributes about 57% of the weight. Including the 2 next most precise studies, the RRp goes from 1.35 to 1.42 to 1.38 and the weight to 76%. With the addition of each of the next 3 most precise studies, the RRp estimate is 1.42. Further addition of the 3 least precise studies gradually brings the RRp back down to 1.33. Thus, if anything, the evidence is somewhat suggestive of an *inverse* relationship between SE and effect size, contrary to what would be expected if publication bias were occurring.

C.4.2. Liver Cancer Effect in the Highest Exposure Groups

C.4.2.1. *Selection of RR Estimates*

The selected RR estimates for liver cancer in the highest TCE exposure categories, for studies that provided such estimates, are presented in Table C-13. Six of the 9 cohort studies reported liver cancer risk estimates categorized by exposure level. As in Section C.4.1.1 for the overall risk estimates, estimates for cancers of the liver and gall bladder/biliary passages combined were preferentially selected, when presented, for the sake of consistency, and, wherever possible, RR estimates for males and females combined were used.

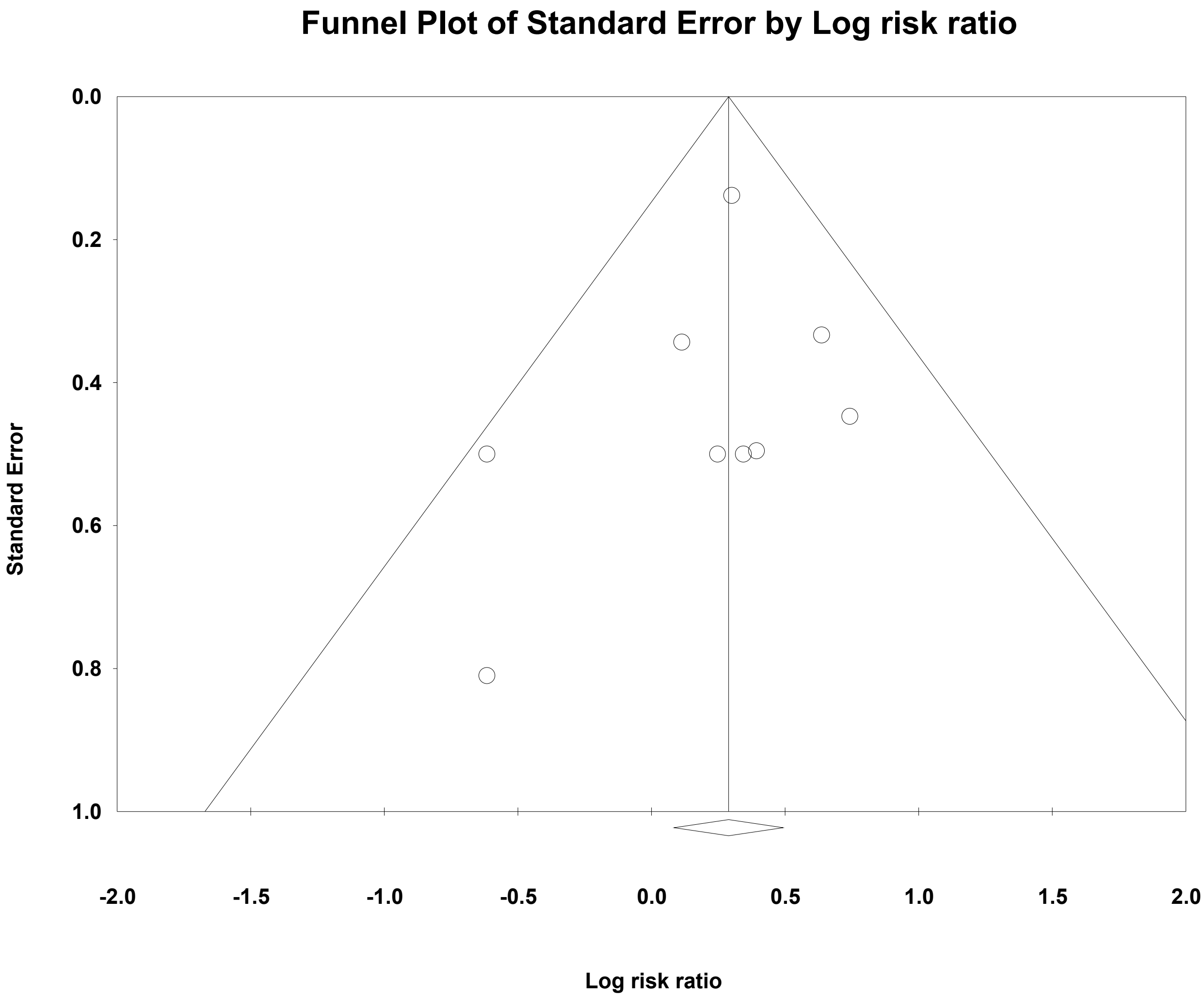


Figure C-10. Funnel plot of SE by log RR estimate for TCE and liver cancer studies.

Table C-13. Selected RR estimates for liver cancer risk in highest TCE exposure groups

Study	RR	95% LCL	95% UCL	Exposure category	log RR	SE(log RR)	Alternate RR estimates	Comments
Anttila et al., 1995	2.74	0.33	9.88	100+ µmol/L U-TCA [a]	1.008	0.707		SIR. ICD-7 155.0 (liver only).
Axelson et al., 1994	3.7	0.09	21	100+ mg/L U-TCA	1.308	1.000	Exclude study	SIR. ICD-7 155. 0 cases observed in highest exposure group (i.e., ≥2 y and 100+ U-TCA), so combined with <2 y and 100+ subgroup and females, estimating the expected numbers (see text).
Boice et al., 1999	0.94	0.36	2.46	≥ 5 yr exposure	−0.062	0.490	None	Mortality RR. ICD-9 155 + 156. For potential routine or intermittent exposure. Adjusted for date of birth, dates 1st and last employed, race, and sex. Referent group is workers not exposed to any solvent.
Hansen et al., 2001				≥ 1080 mos × mg/m^3			1.0 assumed	Reported high exposure group results for some cancer sites but not liver.
Morgan et al., 1998	1.19	0.34	4.16	High cumulative exposure score	0.174	0.639	0.98 (0.29, 3.35) for med/high peak vs. low/no	Mortality RR. ICD-9 155 + 156. Adjusted for age and sex.
Raaschou-Nielsen et al., 2003	1.2	0.7	1.9	≥ 5 yrs	0.182	0.243	1.1 (0.5, 2.1) ICD-7 155.0 (liver only)	SIR. ICD-7 155.0 + 155.1. Male and female results presented separately and combined assuming a Poisson distribution.
Radican et al., 2008	1.49	0.67	3.34	> 25 unit-yr	0.399	0.411	None (see text)	Mortality HR. ICD-8, -9 155 + 156, ICD-10 C22-C24. Male and female results presented separately and combined (see text). Time variable = age, covariate = race. Referent group is workers with no chemical exposures.
Zhao et al., 2005				High exposure score			1.0 assumed	No liver results reported.

[a]Mean personal trichloroacetic acid in urine. 1 µmol/L = 0.1634 mg/L.

Two of the 9 cohort studies (Hansen et al., 2001; Zhao et al., 2005) did not report liver cancer risk estimates categorized by exposure level even though these same studies reported such estimates for selected other cancer sites. To address this reporting bias (as discussed above, Zhao et al. [2005] did not present any liver results, and it is not clear if this was actual reporting bias or an *a priori* decision not to examine liver cancer in the cohort.), attempts were made to obtain the results from the primary investigators, and, failing that, alternate analyses were performed in which null estimates (RR = 1.0) were included for both studies. This alternate analysis was then used as the main analysis, e.g., the basis of comparison for the sensitivity analyses. For the SE (of the logRR) estimates for the null estimates, SE estimates from other sites for which highest-exposure-group results were available were used. For Hansen et al. (2001), the SE estimate for NHL in the highest exposure group was used, because NHL was the only cancer site of interest in this assessment for which highest-exposure-group results were available. For Zhao et al. (2005), the SE estimate for kidney cancer in the highest-exposure group (incidence with 0 lag) was used. (Note that Boice et al. [2006], who studied a cohort that overlapped that of Zhao et al. [2005], also did not present liver cancer results by exposure level.)

For Axelson et al. (1994), there were no liver cancer cases in the highest exposure group (≥2 years and 100+ mean urinary-trichloroacetic acid [U-TCA] level), so no log RR and SE(log RR) estimates were available for the meta-analysis. Instead, the <2 years and ≥2 years results were combined, assuming expected numbers of cases were proportional to person-years, and 100+ U-TCA (with any exposure duration) was used as the highest exposure category. The female contribution to the expected number was also estimated, again assuming proportionality to person-years, and adjusting for the difference between female and male age-adjusted liver cancer incidence rates. The estimated RR and SE values for the combined exposure times and sexes were used in the primary analysis. In an alternate analysis, the Axelson et al. (1994) study was excluded altogether, because we estimated that less than 0.2 cases were expected in the highest-exposure category, suggesting that the study had low power to detect an effect in the highest-exposure group and would contribute little weight to the meta-analysis.

For Boice et al. (1999), only results for workers with “any potential exposure” (rather than “potential routine exposure”) were presented by exposure category, and the referent group is workers not exposed to any solvent. For Morgan et al. (1998), the primary analysis used results for the cumulative exposure metric, and a sensitivity analysis was done with the results for the peak exposure metric. For Raaschou-Nielsen et al. (2003), unlike for NHL and RCC, liver cancer results for the subcohort with expected higher exposure levels were not presented, so the only highest-exposure group results were for duration of employment in the total cohort. Results for cancers of the liver and gall bladder/biliary passages combined were used for the primary analysis and results for liver cancer alone in a sensitivity analysis.

For Radican et al. (2008), it should be noted that the referent group is workers with no chemical exposures, not just no TCE exposure. Furthermore, exposure group results were reported separately for males and females and were combined for this assessment using inverse-variance weighting, as in a fixed effect meta-analysis. In addition to results for biliary passage and liver cancer combined, Radican et al. (2008) present results for liver only by exposure group; however, there were no liver cancer deaths in females and the number expected was not reported, so no alternate analysis for the highest-exposure groups with an RR estimate from Radican et al. (2008) for liver cancer only was conducted. Radican et al. (2008) present only mortality HR estimates by exposure group; however, in an earlier follow-up of this same cohort, Blair et al. (1998) present both incidence and mortality RR estimates by exposure group. As with the Radican et al. (2008) liver cancer only results, however, there were no incident cases for females in the highest-exposure group in Blair et al. (1998) (and the expected number was not reported). Additionally, there were more biliary passage/liver cancer deaths (31) in Radican et al. (2008) than incident cases (13) in Blair et al. (1998) overall and in the highest-exposure group (14 vs. 4). Thus, we elected to use only the Radican et al. (2008) mortality results from this cohort and not to include an alternate analysis based on incidence results from the earlier follow-up.

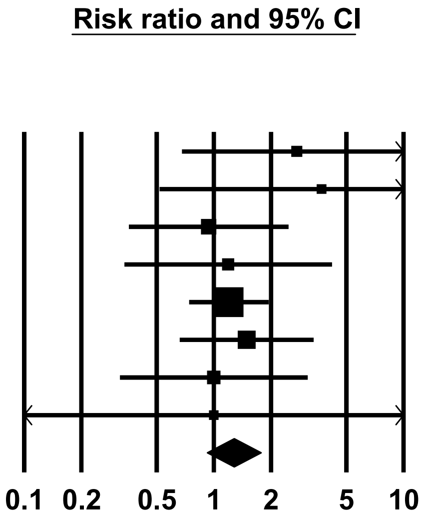
C.4.2.2. *Results of Meta-Analyses*

Results from the meta-analyses that were conducted for liver cancer in the highest exposure groups are summarized at the bottom of Table C-12. The pooled RR estimate from the random effects meta-analysis of the 6 studies with results presented for exposure groups was 1.32 (95% CI: 0.93, 1.86). As with the overall liver cancer meta-analyses, the meta-analyses of the highest-exposure groups were dominated by one study (Raaschou-Nielsen), which provided about 52% of the weight. The RRp estimate from the primary random effects meta-analysis with null RR estimates (i.e., 1.0) included for Hansen and Zhao to address (potential) reporting bias (see above) was 1.28 (95% CI: 0.93, 1.77) (see Figure C-11). The inclusion of these 2 additional studies contributed about 10% of the total weight. No single study was overly influential (removal of individual studies resulted in RRp estimates that ranged from 1.23 to 1.36) and the RRp estimate was not highly sensitive to alternate RR estimate selections (RRp estimates with alternate selections ranged from 1.24 to 1.26; see Table C-12). In addition, there was no observable heterogeneity across the studies for any of the meta-analyses conducted with the highest-exposure groups. However, none of the RRp estimates was statistically significant.

TCE and Liver Cancer - highest exposure groups

Study name	Risk ratio	Lower limit	Upper limit	p-Value
Anttila 1995	2.740	0.685	10.956	0.154
Axelson 1994 est	3.700	0.521	26.267	0.191
Boice 1999	0.940	0.360	2.457	0.900
Morgan 1998	1.190	0.340	4.162	0.785
Raaschou-Nielsen 2003	1.200	0.746	1.930	0.452
Radican 2008	1.490	0.666	3.332	0.331
Hansen 2001	1.000	0.323	3.098	1.000
Zhao 2005	1.000	0.084	11.857	1.000
	1.281	0.925	1.774	0.136

Statistics for each study



random effects model; same for fixed

Figure C-11. Meta-analysis of liver cancer and TCE exposure—highest exposure groups, with assumed null RR estimates for Hansen and Zhao (see text).

Furthermore, the RRp estimates for the highest-exposure groups were all less than the significant RRp estimate for an overall effect on liver cancer (1.33; 95% CI: 1.09, 1.64; see Section C.4.2.2 and Table C-12). This contradictory result is driven by the fact that the RR estimate for the highest-exposure group was less than the overall RR estimate for Raaschou-Nielsen, which contributes the majority of the weight to the meta-analyses. The liver cancer results are relatively underpowered with respect to numbers of studies and number of cases, and the Raaschou-Nielsen study, which dominates the analysis, uses duration of employment as an exposure-level surrogate for liver cancer, and duration of employment is a notoriously weak exposure metric. Thus, the contradictory finding that the RRp estimates for the highest-exposure groups were all less than the RRp estimate for an overall effect does not rule out an effect of TCE on liver cancer; however, it certainly does not provide additional support for such an effect.

C.4.3. Discussion of Liver Cancer Meta-Analysis Results

For the most part, the meta-analyses of the overall effect of TCE exposure on liver (and gall bladder/biliary passages) cancer suggest a small, statistically significant increase in risk. The pooled estimate from the primary random effects meta-analysis of the 9 (all cohort) studies was 1.33 (95% CI: 1.09, 1.64). The analysis was dominated by one large study that contributed about 57% of the weight. When this study was removed, the RRp estimate did not change much, but it was no longer statistically significant (RRp = 1.31; 95% CI: 0.96, 1.79). The pooled estimate was not overly influenced by any other single study, nor was it overly sensitive to individual RR estimate selections. The largest downward impacts were from the removal of the Anttila study, resulting in an RRp estimate of 1.29 (95% CI: 1.04, 1.59), and from the substitution of the Boice (1999) RR estimate for potential routine exposure with that for any potential exposure, resulting in an RRp estimate of 1.29 (1.06, 1.56). Substituting the RR estimates for liver/gall bladder/biliary passage cancers with those of liver cancer alone for the 3 studies that provided these results yielded an RRp estimate of 1.31 (1.02, 1.67). There was no evidence of publication bias in this data set, and there was no observable heterogeneity across the study results.

Six of the 9 studies provided liver cancer results by exposure level. Two other studies reported results for other cancer sites by exposure level, but not liver cancer; thus, to address this reporting bias, null values (i.e., RR estimates of 1.0) were used for these studies. Different exposure metrics were used in the various studies, and the purpose of combining results across the different highest exposure groups was not to estimate an RRp associated with some level of exposure, but rather to see the impacts of combining RR estimates that should be less affected by exposure misclassification. In other words, the highest exposure category is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any vs. none) exposure comparison. Thus, if TCE exposure increases the risk of liver cancer, the effects should be more apparent in the highest exposure groups. However, the RRp estimate from the meta-analyses of the highest exposure group results were less than the RRp estimate from the overall exposure analysis. This anomalous result is driven by the fact that, for Raaschou-Nielsen, which contributes the majority of the weight to the meta-analyses, the RR estimate for the highest-exposure group, although greater than 1.0, was less than the overall RR estimate.

Thus, while there is the suggestion of an increased risk for liver cancer associated with TCE exposure, the statistical significance of the pooled estimates is dependent on one study, which provides the majority of the weight in the meta-analyses. Removal of this study does not change the RRp estimate; however, it becomes nonsignificant ($p = 0.08$). Furthermore, meta-analysis results for the highest-exposure groups yielded *lower* RRp estimates than for an overall effect. These results do not rule out an effect of TCE on liver cancer, because the liver cancer results are relatively underpowered with respect to numbers of studies and number of cases and the overwhelming study in terms of weight uses the weak exposure surrogate of duration of employment for categorizing exposure level; however, at present, there is only modest support for such an effect.

C.5. DISCUSSION OF STRENGTHS, LIMITATIONS, AND UNCERTAINTIES IN THE META-ANALYSES

Meta-analysis provides a systematic way of objectively and quantitatively combining the results of multiple studies to obtain a summary effect estimate. Use of meta-analysis can help risk assessors avoid some of the potential pitfalls in overly relying on a single study or in making more subjective qualitative judgments about the apparent weight of evidence across studies. Combining the results of smaller studies also increases the statistical power to observe an effect, if one exists. In addition, meta-analysis techniques assist in systematically investigating issues such as potential publication bias and heterogeneity in a database.

While meta-analysis can be a useful tool for analyzing a database of epidemiological studies, the analysis is limited by the quality of the input data. If the individual studies are deficient in their abilities to observe an effect (in ways other than low statistical power, which meta-analysis can help ameliorate), the meta-analysis will be similarly deficient. A critical step in the conduct of a meta-analysis is to establish eligibility criteria and clearly and transparently identify all relevant studies for inclusion in the meta-analysis. For the TCE database, a comprehensive qualitative review of available studies was conducted and eligible studies were identified, as described in Appendix B, Section II-9.

Identifying all relevant studies may be hampered if publication bias has occurred. Publication bias is a systematic error that can arise if statistically significant studies are more likely to be published than nonsignificant studies. This can result in an upward bias on the effect size measure, i.e., the relative risk estimate. To address this concern, potential publication bias was investigated for the databases for which meta-analyses were undertaken. For the studies of kidney cancer and liver cancer, there was no evidence of publication bias. For the studies of lymphoma, there was some evidence of potential publication bias. It is uncertain whether this reflects actual publication bias or rather an association between SE and effect size (as discussed in Section C.1, a feature of publication bias is that smaller studies tend to have larger effect sizes) resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias in this data set, it may be creating an upward bias on the relative risk estimate, but this bias does not appear to account completely for the finding of an increased lymphoma risk (see Section C.2.1.2).

Another concern in meta-analyses is heterogeneity across studies. Random-effects models were used for the primary meta-analyses in this assessment because of the diverse nature of the individual studies. When there is no heterogeneity across the study results, the random-effects model will give the same result as a fixed-effect model. When there is heterogeneity, the random-effects model estimates the between-study variance. Thus, when there is heterogeneity, the random-effects model will generate wider confidence intervals and be more "conservative" than a fixed-effect model. However, if there is substantial heterogeneity, it may be inappropriate to combine the studies at all. In cases of significant heterogeneity, it is important to try to investigate the potential sources of the heterogeneity.

For the studies of kidney cancer and liver cancer, there was no apparent heterogeneity across the study results, i.e., random- and fixed-effects models gave identical summary estimates. For the lymphoma studies, there was heterogeneity, but it was not statistically significant ($p = 0.10$). The I^2 value was 33%, suggesting low-to-moderate heterogeneity. When subgroup analyses were done for the cohort and case-control studies separately, there was some heterogeneity in both groups, but in neither case was it statistically significant. Further attempts to quantitatively investigate the heterogeneity were not pursued because of limitations in the database. The sources of heterogeneity are an uncertainty in the database of studies of TCE and lymphoma. Some potential sources of heterogeneity, which are discussed qualitatively in Section C.2.3, include differences in exposure assessment or in the intensity or prevalence of TCE exposures in the study population and differences in lymphoma classification.

The joint occurrence of heterogeneity and potential publication bias in the database of studies of TCE and lymphoma raises special concerns. Because of the heterogeneity, a random-effects model should be used if these studies are to be combined; yet, the random-effects model gives relatively large weight to small studies, which could exacerbate the potential impacts of publication bias. For the lymphoma studies, the summary relative risk estimates from the random-effects and fixed-effect models are not very different (RRp = 1.23 [95% CI: 1.04, 1.44] and 1.19 [1.06, 1.34], respectively); however, the confidence interval for the fixed-effect estimate does not reflect the between-study variance and is, thus, overly narrow.

C.6. CONCLUSIONS

The strongest finding from the meta-analyses was for TCE and kidney cancer. The summary estimate from the primary random-effects meta-analysis of the 14 studies was RRp = 1.25 (95% CI: 1.11, 1.41). There was no apparent heterogeneity across the study results (i.e., fixed-effect model gave same summary estimate), and there was no evidence of potential publication bias. The summary estimate was robust across influence and sensitivity analyses; the estimate was not markedly influenced by any single study, not was it overly sensitive to individual RR estimate selections. The findings from the meta-analyses of the highest exposure groups for the studies that provided results categorized by exposure level were similarly robust. The summary estimate was RRp = 1.53 (95% CI: 1.23, 1.91) for the 12 studies included in the analysis. There was no apparent heterogeneity in the highest-exposure group results, and the estimate was not markedly influenced by any single study, nor was it overly sensitive to individual RR estimate selections. In sum, these robust results support a conclusion that TCE exposure increases the risk of kidney cancer.

For the most part, the meta-analyses of the overall effect of TCE exposure on lymphoma also suggest a small, statistically significant increase in risk. The summary estimate from the primary random-effects meta-analysis of the 16 studies was 1.23 (95% CI: 1.04, 1.44). This result was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections, although use of one alternate RR estimate considered clearly inferior narrowly eliminated statistical significance of the summary estimate ($p = 0.050$). There is some evidence of potential publication bias in the lymphoma study data set; however, it is uncertain that this is actually publication bias rather than an association between SE and effect size resulting for some other reason, e.g., a difference in study populations or protocols in the smaller studies. Furthermore, if there is publication bias, it does not appear to account completely for the findings of an increased lymphoma risk. There was some heterogeneity across the results of the 16 studies, but it was not statistically significant ($p = 0.10$). The I^2 value was 33%, suggesting low-to-moderate heterogeneity. The source(s) of this heterogeneity remains an uncertainty. The summary estimate from the meta-analysis of the highest exposure groups for the 12 studies which provided results categorized by exposure level was RRp = 1.57 (95% CI: 1.27, 1.94). This result for the highest exposure groups was not overly influenced by any single study, nor was it overly sensitive to individual RR estimate selections, and heterogeneity was not observed in any of the relevant analyses. The robustness of the finding of an increased lymphoma risk for the highest exposure groups strengthens the more moderate evidence from the meta-analyses for overall effect.

The meta-analyses of the overall effect of TCE exposure on liver (and gall bladder/biliary passages) cancer also suggest a small, statistically significant increase in risk, but the study database is more limited. The pooled estimate from the primary random-effects meta-analysis of the 9 (all cohort) studies was 1.33 (95% CI: 1.09, 1.64). The analysis was dominated by one large study that contributed about 57% of the weight. When this study was removed, the RRp estimate did not change much, but it was less precise (RRp = 1.31; 95% CI: 0.96, 1.79). The pooled estimate was not overly influenced by any other single study, nor was it overly sensitive to individual RR estimate selections. There was no evidence of publication bias in this data set, and there was no observable heterogeneity across the study results. However, the findings from the meta-analyses of the highest-exposure groups for the studies that provided results categorized by exposure level do not add support to the overall effect findings. The summary estimate was RRp = 1.28 (95% CI: 0.93, 1.77) for the 8 studies included in the analysis, which is *lower* than the summary estimate for the overall effect. This contradictory result is driven by the fact that the RR estimate for the highest-exposure group in the individual study which contributes the majority of the weight to the meta-analyses, although greater than 1.0, was less than the overall RR estimate for the same study. In sum, these results do not rule out an effect of TCE on liver cancer, because the liver cancer results are relatively underpowered with respect to numbers of studies and number of cases and the overwhelming study in terms of weight uses the weak exposure surrogate of duration of employment for categorizing exposure level; however, at present, there is only modest support for an increased risk of liver cancer.

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APPENDIX D

Neurological Effects of Trichloroethylene

CONTENTS—Appendix D: Neurological Effects of Trichloroethylene

LIST OF TABLES

APPENDIX D. NEUROLOGICAL EFFECTS OF TRICHLOROETHYLENE

D.1. HUMAN STUDIES ON THE NEUROLOGICAL EFFECTS OF TRICHLOROETHYLENE (TCE)

There is an extensive body of evidence in the literature on the neurological effects caused by exposure to trichloroethylene (TCE) in humans. The primary functional domains that have been studied and reported are trigeminal nerve function and nerve conductivity (latency), psychomotor effects, including reaction times (simple and choice), visual and auditory effects, cognition, memory, and subjective neurological symptoms, such as headache and dizziness. This section discusses the primary studies presented for each of these effects. Summary tables for all the human TCE studies are at the end of this section.

D.1.1. Changes in Nerve Conduction

There is strong evidence in the literature that exposure to TCE results in impairment of trigeminal nerve function in humans exposed occupationally, by inhalation, or environmentally, by ingestion. Functional measures such as the blink reflex and masseter reflex tests were used to determine if physiological functions mediated by the trigeminal nerve were significantly impacted. Additionally, trigeminal somatosensory evoked potentials were also measured in some studies to ascertain if nerve activity was directly affected by TCE exposure.

D.1.1.1. *Blink Reflex and Masseter Reflex Studies—Trigeminal Nerve*

Barret et al. (1984) conducted a study on 188 workers exposed to TCE occupationally from small and large factories in France (type of factories not disclosed). The average age of the workers was 41 (standard deviation [SD] not provided, but authors noted 14% <30 years and 25% >50 years) and the average exposure duration was 7 hours/day for 7 years. The 188 workers were divided into high and low exposure groups for both TCE exposure measured using detector tubes and trichloroacetic acid (TCA) levels measured in urine. There was no unexposed control population, but responses in the high-exposure group were compared response in the low-exposure group. TCE exposure groups were divided into a low exposure group (<150 ppm; n = 134) and a high exposure group (>150 ppm; n = 54). The same workers (n = 188) were also grouped by TCA urine measurements such that a high exposure was ≥100 mg TCA/g creatinine. Personal factors including age, tobacco use and alcohol intake were also analyzed. No mention was made regarding whether or not the examiners were blind to the subjects' exposure status. Complete physical examination including testing visual performance (acuity and color perception), evoked trigeminal potential latencies and audiometry, facial sensitivity, reflexes, and motoricity of the masseter muscles. Chi squared analysis was used to examine distribution of the different groups for comparing high and low exposed workers followed by one way analysis of variance. Overall, 22 out of 188 workers (11.7%) experienced trigeminal nerve impairment ($p < 0.01$) as measured by facial sensitivity, reflexes (e.g., jaw, corneal, blink) and movement of the masseter muscles. When grouped by TCE exposure, 12 out of 54 workers (22.2%) in the high exposure group (≥150 ppm) and 10 out of 134 workers (7.4%) in the low exposure group had impaired trigeminal nerve mediated responses. When grouped by the presence of TCA in the urine, 41 workers were now in the high TCA group and 10 out of 41 workers (24.4%) experienced trigeminal nerve impairment in comparison to the 12 out of 147 (8.2%) in the low TCA (<100 mg TCA/g creatinine) group. Statistically significant results were also presented for the following symptoms based on TCE and TCA levels: trigeminal nerve impairment ($p < 0.01$), asthenia ($p < 0.01$), optic nerve impairment ($p < 0.001$), and dizziness ($0.05 < p < 0.06$). Statistically significant results were also presented for the following symptoms based on TCA levels: Trigeminal nerve impairment ($p < 0.01$), asthenia ($p < 0.01$), optic nerve impairment ($p < 0.001$), headache ($p < 0.05$), and dizziness ($0.05 < p < 0.06$). Symptoms for which there is a synergistic toxic role for TCE and alcohol ($p < 0.05$) were liver impairment and degreaser flush. This study presents a good statistically significant dose-response relationship between TCE/TCA exposure and trigeminal nerve impairment. TCE concentrations are not available for individual subjects, but exposure assessment was inferred based on occupational standards at the time of the study.

Feldman et al. (1988) conducted an environmental study on 21 Woburn, Massachusetts residents with alleged chronic exposure to TCE in drinking water, resulting from an environmental spill by a local industry. These were from 8 families whose drinking water wells were found to be contaminated with TCE and other solvents. The subjects were self selected, having been referred for clinical evaluation due to suspected neurotoxicity, and were involved in litigation. The control group was 27 unexposed residents from a nearby community with TCE concentrations in drinking water below state standards. TCE in residential well water was measured over a prior 2 year period (1979−1981); the maximum reported concentration for the study population was 267 ppb. The residents' water supply came from two different TCE-contaminated wells that had an average measured concentration of 256 ppb (labeled "Well G" based on 6 samples) and 111 ppb (labeled "Well H;" based on 4 samples). The residents' exposure ranged from 1−12 years and was dependent on the length of residence and the age of the subject. There were other solvents found to be present in the well water, and TCE data were not available for the entire exposure period. TCE concentrations for the control population were less than the maximum contaminant level (MCL) (5 ppb). The BR was used to measure the neurotoxic effects of TCE. The BR was measured using an electrode to stimulate the supraorbital nerve (above the eyelid) with a shock (0.05 ms in duration) resulting in a response and the response was measured using a recording electrode over the orbicularis oculi muscle (the muscle responsible for closing the eyelid and innervated by the trigeminal nerve). The BR generated an R1 and an R2 component from each individual. BRs were recorded and the supraorbital nerve was stimulated with single electrical shocks of increasing intensity until nearly stable R1 and R2 ipsilateral and R2 contralateral responses were obtained. The student's t-test was used for testing the difference between the group means for the blink reflex component latencies. Because of the variability of R2 responses, this study focused primarily on the R1 response latencies. Highly significant differences in the conduction latency means of the BR components for the TCE exposed population versus control population were observed when comparing means for the right and left side R1 to the controls. The mean R1 BR component latency for the exposed group was 11.35 ms, SD = 0.74 ms, 95% confidence interval (CI): 11.03−11.66. The mean for the controls was 10.21 ms, SD = 0.78 ms, 95% CI: 9.92−10.51; ($p < 0.001$). The study was well conducted with consistency of methods, and statistically significant findings for trigeminal nerve function impairment resulting from environmental exposures to TCE. However, the presence of other solvents in the well water, self selection of subjects involved in litigation, and incomplete characterization of exposure present problems in drawing a clear conclusion of TCE causality or dose-response relationship.

Kilburn and Warshaw (1993) conducted an environmental study on 544 Arizona residents exposed to TCE in well-water. TCE concentrations were from 6 to 500 ppb and exposure ranged from 1 to 25 years. Subjects were recruited and categorized in 3 groups. Exposed group 1 consisted of 196 family members with cancer or birth defects. Exposed group 2 consisted of 178 individuals from families without cancer or birth defects; and exposed group 3 included 170 parents whose children had birth defects and rheumatic disorders. Well-water was measured from 1957 to 1981 by several governmental agencies and average annual TCE exposures were calculated and then multiplied by each individual's years of residence for 170 subjects. A referent group of histology technicians ($n = 113$) was used as a comparison for the BR test. For this test, recording electrodes were placed over the orbicularis oculi muscles (upper and lower) and the BR was elicited by gently tapping the glabeela (located on the mid-frontal bone at the space between the eyebrows and above the nose). A two-sided Student's t-test and linear regression were used for statistical analysis. Significant increases in the R1 component of the BR response was observed in the exposed population as compared to the referent group. The R1 component measured from the right eye appeared within 10.9 ms in TCE-exposed subjects whereas in referents, this component appeared 10.2 ms after the stimulus was elicited indicating a significant delay ($p < 0.008$) in the reflex response. Similarly, delays in the latency of appearance for the R1 component were also noted for the left eye but the effect was not statistically significant ($p = 0.0754$). This study shows statistically significant differences in trigeminal nerve function between subjects environmentally exposed and nonexposed to TCE. This is an ecological study with TCE exposure inferred to subjects by residence in a geographic area. Estimates of TCE concentrations in drinking water to individual subjects are lacking. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Kilburn (2002a) studied 236 residents (age range: 18−83 years old) lived nearby manufacturing plants (e.g., microchip plants) in Phoenix, AZ. Analysis of the groundwater in the residential area revealed contamination with many volatile organic compounds including TCE. Concentrations of TCE in the well water ranged from 0.2 ppb to more than 10,000 ppb and the exposure duration varied between 2 to 37 years. Additional associated solvents included dichloroethane (DCE), perchloroethylene, and vinyl chloride. A group-match design was used to compare the 236 TCE-exposed residents to 161 unexposed regional referents and 67 referents in NE Phoenix in the BR test. The BR response was recorded from surface electrodes placed over the location of the orbicularis oculi muscles. The reflex response was elicited by gently tapping the left and right supraorbital notches with a small hammer. The R1 component of the BR response was measured for both the left and right eye. Statistically significant increases in latency time for the R1 component was observed for residents exposed to TCE in comparison to the control groups. In unexposed individuals, the R1 component occurred within 13.4 ms from the right eye and 13.5 ms from the left eye. In comparison, the residents near the manufacturing plant had latency times of 14.2 ms ($p < 0.0001$) for the right eye and 13.9 ms ($p < 0.008$) for the left eye. This study shows statistically significant differences between environmentally exposed and unexposed populations for trigeminal nerve function, as a result of exposures to TCE. This is an ecological study with TCE exposure potential to subjects inferred by residence in a geographic area. Estimates of TCE concentrations in drinking water to individuals are lacking. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Feldman et al. (1992) evaluated the BR reflex in 18 subjects occupationally exposed to neurotoxic chemicals (e.g., degreasers, mechanics, and pesticide sprayers among many others). Eight of the subjects were either extensively ($n = 4$) or occupationally ($n = 4$) exposed to TCE. The remaining subjects ($n = 10$) were exposed to other neurotoxic chemicals, but not TCE. Quantitative exposure concentration data were not reported in the study, but TCE exposure was characterized as either "extensive" or "occupational." Subjects in the "extensive" exposure group were chronically exposed (≥1 year) to TCE at least 5 days a week and for at greater than 50% of the workday ($n = 3$) or experienced a direct, acute exposure to TCE for greater than 15 minutes ($n = 1$). Subjects in the "occupational" group were chronically exposed (≥1 year) to

TCE for 1−3 days/week and for greater than 50% of the workday. The BR responses from the TCE-exposed subjects were compared to a control group consisting of 30 nonexposed subjects with no noted neurological disorders. BR responses were measured using surface electrodes over the lower lateral portion of the orbicularis oculi muscle. Electrical shocks with durations of 0.05 ms were applied to the supraorbital nerve to generate the R1 and R2 responses. All of the subjects that were extensively exposed to TCE had significantly increased latency times in the appearance of the R1 component (no *p*-value listed) and for 3 subjects this increased latency time persisted for at least 1 month and up to 20 years postexposure. However, none of the subjects occupationally exposed to TCE had changes in the BR response in comparison to the control group. In comparing the remaining neurotoxicant exposed subjects to the TCE-exposed individuals, the sensitivity, or the ability of a positive blink reflex test to identify correctly those who had TCE exposure was 50%. However, in workers with no exposure to TCE, 90% demonstrated a normal R1 latency.

Mixed results were obtained in a study by Ruitjen et al. (1991) on 31 male printing workers exposed to TCE. The mean age was 44; mean exposure duration was 16 years and had at least 6 years of TCE exposure. The control group consisted of 28 workers with a mean age 45 years. Workers in the control group were employed at least 6 years in print factories (similar to TCE-exposed), had no exposure to TCE, but were exposed to "turpentine-like organic solvents." TCE exposure potential was inferred from historical monitoring of TCE at the plant using gas detection tubes. These data indicated TCE concentrations in the 1960s of around 80 ppm, mean concentration of 70 ppm in the next decade, with measurements from 1976 and 1981 showing a mean concentration of 35 ppm. The most recent estimate of TCE concentrations in the factory was 17 ppm (stable for 3 years) at the time of the report. The authors calculated that mean cumulative TCE exposure would be 704 ppm × years worked in factory. The masseter and blink reflexes were measured to evaluate trigeminal nerve function in TCE-exposed and control workers. For measurement of the masseter reflex, surface electrodes were attached over the right masseter muscle (over the cheek area). A gentle tap on a roller placed under the subject's chin was used to elicit the masseter reflex. For measurement of the blink reflex, surface electrodes were placed on the muscle near the upper eyelid. Electrical stimulation of the right supraorbital nerve was used to generate the blink reflex. There was a significant increase in the latency of the masseter reflex to appear for the TCE-exposed workers ($p < 0.05$). However, there was no significant change in the blink reflex measure between TCE-exposed workers and control. Although no change in the blink reflex measures were observed between the two groups, it should be noted that the control group was exposed to other volatile organic solvents (not specified) and this volatile organic compound exposure could be a possible confounder for determination of TCE-induced effects.

There are two studies that reported no effect of TCE exposure on trigeminal nerve function (El-Ghawabi et al., 1973; Rasmussen et al., 1993c). El-Ghawabi et al. (1973) conducted a study on 30 money printing shop workers occupationally exposed to TCE. Metabolites of total trichloroacetic acid and trichloroethanol were found to be proportional to TCE concentrations up to 100 ppm (550 mg/m^3). Controls were 20 age- and socio-economic status (SES)-matched nonexposed males and 10 control workers not exposed to TCE. Trigeminal nerve involvement was not detected, but the authors failed to provide details as to how this assessment was made. It is mentioned that each subject was clinically evaluated and trigeminal nerve involvement may have been assessed through a clinical evaluation. As a result, the conclusions of this study are tempered since the authors did not provide details as to how trigeminal nerve function was evaluated in this study.

Rasmussen et al. (1993c) conducted an historical cohort study on 99 metal degreasers. Subjects were selected from a population of 240 workers from 72 factories in Denmark. The participants were divided into three groups based on solvent exposure durations where low exposure was up to 0.5 years, medium was 2.1 years and high was 11.0 years (mean exposure duration). Most of the workers (70 out of 99) were primarily exposed to TCE with an average exposure duration of 7.1 years for 35 hours/week. TCA and trichloroethanol (TCOH) levels were measured in the urine samples provided by the workers and mean TCA levels in the high group was 7.7 mg/L and was as high as 26.1 mg/L. Experimental details of trigeminal nerve evaluation were not provided by the authors. It was reported that 1 out of 21 people (5%) in the low exposure, 2 out of 37 (5%) in the medium exposure and 4 out of 41 (10%) in the high exposure group experienced abnormalities in trigeminal nerve sensory function. No linear association was seen on trigeminal nerve function (Mantel-Haenzel test for linear association, $p = 0.42$). However, the trigeminal nerve function findings were not compared to a control (no TCE exposure) group and it should be noted that some of the workers (29 out of 99) were not exposed to TCE.

D.1.1.2. *Trigeminal Somatosensory Evoked Potential (TSEP) Studies—Trigeminal Nerve*

In a preliminary study, Barret et al. (1982) measured trigeminal sensory evoked potentials (TSEPs) in eleven workers that were chronically exposed to TCE. Nine of these workers were suffering effects from TCE intoxication (changes in facial sensitivity and clinical changes in trigeminal nerve reflexes), and two were TCE-exposed without exhibiting any clinical manifestations from exposure. A control group of 20 nonexposed subjects of varying ages were used to establish the normal response curve for the trigeminal nerve function. In order to generate a TSEP, a surface electrode was placed over the lip and a voltage of 0.05 ms in duration was applied. The area was stimulated 500 times at a rate of two times per second. TSEPs were recorded from a subcutaneous electrode placed between the international CZ point (central midline portion of the head) and the ear. In eight of the eleven workers, an increased voltage ranging from a 25 to a 45 volt increase was needed to generate a normal TSEP. Two of the 11 workers had an increased latency of appearance for the TSEP and three workers had increases in TSEP amplitudes. The preliminary findings indicate that TCE exposure results in abnormalities in trigeminal nerve function. However, the study does not provide any exposure data and lacks information with regards to the statistical treatment of the observations.

Barret et al. (1987) conducted a study on 104 degreaser machine operators in France (average age = 41.6 years; range = 18−62 years) who were highly exposed to TCE with an average exposure of 7 hours/day for 8.23 years. Although TCE exposure concentrations were not available, urinary concentrations of TCOH and TCA were measured for each worker. A control group consisting of 52 subjects without any previous solvent exposure and neurological deficits was included in the study. Trigeminal nerve symptoms and TSEPs were collected for each worker. Trigeminal nerve symptoms were clinically assessed by examining facial sensitivity and reflexes dependent on this nerve such as the jaw and blink reflex. TSEPs were elicited by electrical stimulation (70−75 V for 0.05 ms) of the nerve using an electrode on the lip commissure. Eighteen out of 104 TCE-exposed machine operators (17.3%) had trigeminal nerve symptoms. The subjects that experienced trigeminal nerve symptoms were significantly older (47.8 years vs. 40.5; $p < 0.001$). Both groups had a similar duration of exposure with a mean of 9.2 years in the sensitive group and 7.8 years in the nonsensitive group. Urinary concentrations of TCOH and TCA were also statistically similar although the levels were slightly higher in the sensitive group (245 mg/g creatinine vs. 162 mg/g creatinine for TCOH; 131 mg/g creatinine vs. 93 mg/g creatinine for TCA). However, in the same group, 40 out of 104 subjects (38.4%) had an abnormal TSEP. Abnormal TSEPs were characterized as potentials that exhibited changes in latency and/or amplitude that were at least 2.5 times the standard deviation of the normal TSEPs obtained from the control group. Individuals with abnormal TSEP were significantly older (45 years vs. 40.1 years; $p < 0.05$) and were exposed to TCE longer (9.9 years vs. 5.6 years; $p < 0.01$). Urinary concentrations TCOH and TCA were similar between the groups with sensitive individuals having average metabolite levels of 195 mg TCOH/g creatinine and 98.3 mg TCA/g creatinine in comparison to 170 mg TCOH/g creatinine and 96 mg TCA/g creatinine in nonsensitive individuals. When a comparison was made between workers that had normal TSEP and no trigeminal symptoms and workers that had an abnormal TSEP and experienced trigeminal symptoms, it was found that in the sensitive individuals (abnormal TSEP and trigeminal symptoms) there was a significant increase in age (48.5 vs. 39.5 years old, $p < 0.01$), duration of exposure (11 vs. 7.5 years, $p < 0.05$) and an increase in urinary TCA (313 vs. 181 mg TCA/g creatinine). No significant changes were noted in urinary TCOH, but the levels were slightly higher in sensitive individuals (167 vs. 109 mg TCOH/g creatinine). Overall, it was concluded that abnormal TSEPs were recorded in workers who were exposed to TCE for a longer period (average duration 9.9 years). This appears to be a well designed study with statistically significant results reported for abnormal trigeminal nerve response in TCE exposed workers. Exposure assessment to TCE is by exposure duration and mean urinary TCOH and TCA concentrations. TCE concentrations to exposed subjects as measured by atmospheric or personal monitoring are lacking.

Mhiri et al. (2004) measured TSEPs from 23 phosphate industry workers exposed to TCE for 6 hours/day for at least two years while cleaning tanks. Exposure assessment was based on measurement of urinary metabolites of TCE, which were performed 3 times/worker, and air measurements. Blood tests and hepatic enzymes were also collected. The mean exposure duration was 12.4 ± 8.3 years (exposure duration range = 2−27 years). Although TCE exposures were not provided, mean urinary concentrations of TCOH, TCA, and total trichlorides were 79.3 ± 42, 32.6 ± 22, and 111.9 ± 55 mg/g urinary creatinine, respectively. The control group consisted of 23 unexposed workers who worked in the same factory without being exposed to any solvents. TSEPs were generated from a square wave pulses (0.1 ms in duration) delivered through a surface electrode that was placed 1 cm under the corner of the mouth. The responses to the stimuli (TSEPs) were recorded from another surface electrode that was placed over the contralateral parietal area of the brain. The measured TSEP was divided into several components and labeled according to whether it was (1) a positive (P) or negative (N) potential and (2) the placement of the potential in reference to the entire TSEP (e.g., P1 is the first positive potential in the TSEP). TSEPs generated from the phosphate workers that were ± 2.5 times the standard deviation from the TSEPs obtained from the control group were considered abnormal. Abnormal TSEP were observed in 6 workers with clinical evidence of trigeminal involvement and in 9 asymptomatic workers. Significant increases in latency were noted for all TSEP potentials (N1, P1, N2, P2, N3, $p < 0.01$) measured from the phosphate workers. Additionally, significant decreases in the P1 ($p < 0.02$) and N2 ($p < 0.05$) amplitudes were observed. A significant positive correlation was demonstrated between duration of exposure and the N2 latency ($p < 0.01$) and P2 latency ($p < 0.02$). Only one subject had urinary TCE metabolite levels over tolerated limits. TCE air contents were over tolerated levels, ranging from 50−150 ppm (275−825 mg/m^3). The study is well presented with statistically significant results for trigeminal nerve impairment resulting from occupational exposures to TCE. Exposure potential to TCE is defined by urinary biomarkers, TCA, total trichloro-compounds, and TCOH. The study lacks information on atmospheric monitoring of TCE in this occupational setting.

D.1.1.3. *Nerve Conduction Velocity Studies*

Nerve conduction latencies were also studied in two occupational studies by Triebig et al. (1982, 1983) using methods for measurement of nerve conduction which differ from most published studies, but the results indicate a potential impact on nerve conduction following occupational TCE exposure. There was no impact seen on latencies in the 1982 study, but a statistically significant response was observed in the latter study. The latter study, however, is confounded by multiple solvent exposures.

In Triebig et al. (1982), 24 healthy workers (20 males, 4 females) were exposed to TCE occupationally at three different plants. The ages ranged from 17−56, and length of exposure ranged from 1 month to 258 months (mean 83 months). TCE concentrations measured in air at work places ranged from 5−70 ppm (27−385 mg/m^3). A control group of 144 healthy, complaint-free individuals were used to establish 'normal' responses on the nerve conduction studies. The matched control group consisted of twenty-four healthy nonexposed individuals (20 males, 4 females), chosen to match the subjects for age and sex. TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine. Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV_{MAX} (U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U): Distal NLG of mixed fibers of the N. ulnaris between finger V and the wrist joint; pSCV (U): Proximal NLG of sensory fibers of the N. medianus between finger V and Sulcus ulnaris; and dSCV (M): Distal NLG of sensory fibers of the N. medianus between finger III and the wrist joint. Data were analyzed using parametric and nonparametric tests, rank correlation, linear regression, with 5% error probability. Results show no statistically significant difference in nerve conduction velocities between the exposed and unexposed groups. This study has measured exposure data, but exposures/responses are not reported by dose levels.

Triebig et al. (1983) has a similar study design to the previous study (Triebig et al., 1982) in the tests used for measurement of nerve conduction velocities, and in the analysis of blood and urinary metabolites of TCE. However, in this study, subjects were exposed to a mixture of solvents, including TCE, specifically "ethanol, ethyl acetate, aliphatic hydrocarbons (gasoline), methyl ethyl ketone (MEK), toluene, and trichloroethene." The exposed group consists of 66 healthy workers selected from a population of 112 workers. Workers were excluded based on polyneuropathy (n = 46) and alcohol consumption (n = 28). The control group consisted of 66 healthy workers with no exposures to solvents. Subjects were divided into three exposure groups based on length of exposure, as follows: 20 employees with "short-term exposure" (7−24 months); 24 employees with "medium-term exposure" (25−60 months); 22 employees with "long-term exposure" (over 60 months). TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine. Subjects were divided into exposure groups based on length of exposures, and results were compared for each exposure group to the control group. In this study, there was a dose-response relationship observed between length of exposure to mixed solvents and statistically significant reduction in nerve conduction velocities observed for the medium and long-term exposure groups for the ulnar nerve (NCV). Interpretation of this study is limited by the mixture of solvent exposure, with no results reported for TCE alone.

D.1.2. Auditory Effects

There are three large environmental studies reported which assessed the potential impact of TCE exposures through groundwater ingestion on auditory functioning. They present mixed results. All three studies were conducted on the population in the TCE Subregistry from the National Exposure Registry (NER) developed by the Agency for Toxic Substances Disease Registry (ATSDR). The two studies conducted by Burg et al. (1995; Burg and Gist, 1999) report an increase in auditory effects associated with TCE exposure, but the auditory endpoints were self reported by the population, as opposed to testing of measurable auditory effects in the subject population. The third of these studies, reported by ATSDR (2003) conducted measurements of auditory function on the subject population, but failed to demonstrate a positive relationship between TCE exposure and auditory effects. Results from these studies strongly suggest that children ≤9 years are more susceptible to hearing impairments from TCE exposure than the rest of the general population. These studies are described below.

Burg et al. (1995) conducted a study on registrants in the National Health Interview Survey (NHIS) TCE subregistry of 4,281 (4,041 living and 240 deceased) residents environmentally exposed to TCE via well water in Indiana, Illinois, and Michigan. Morbidity baseline data were examined from the TCE Subregistry from the NER developed by the ATSDR. Participants were interviewed in the NHIS, which consists of 25 questions about health conditions. Data were self reported via face-to-face interviews. Neurological endpoints were hearing and speech impairments. This study assessed the long-term health consequences of long-term, low-level exposures to TCE in the environment. The collected data were compared to the NHIS, and the National Household Survey on Drug Abuse. Poisson Regression analysis model was used for registrants 19 and older. The statistical analyses performed treated the NHIS population as a standard population and applied the age- and sex-specific period prevalence and prevalence rates obtained from the NHIS data to the corresponding age- and sex-specific denominators in the TCE Subregistry. This one-sample approach ignored sampling variability in the NHIS data because of the large size of the NHIS database when compared to the TCE Subregistry data file. A binomial distribution was assumed in estimating standard errors for the TCE Subregistry data. Weighted age- and sex-specific period prevalence and prevalence rates by using the person-weights were derived for the TCE subregistry. These "standard" rates were applied to the corresponding TCE Subregistry denominators to obtain expected counts in each age and sex combination. In the NHIS sample, 18% of the subjects were nonwhite. In the TCE Subregistry sample, 3% of the subjects were nonwhite. Given this discrepancy in the proportion of nonwhites and the diversity of races reported among the nonwhites in the TCE Subregistry, the statistical analyses included 3,914 exposed white TCE registrants who were alive at baseline. TCE registrants that were 9 years old or younger had a statistically significant increase in hearing impairment as reported by the subjects. The relative risk (RR) in this age group for hearing impairments was 2.13. The RR decreased to 1.12 for registrants aged 10−17 years and to 0.32 or less for all other age groups. As a result, the effect magnitude was lower for children 10−17 years and for all other age groups. The study reports a dose-response relationship, but the hearing effects are self-reported, and exposure data are modeled estimates.

Burg and Gist (1999) reported a study conducted on the same subregistry population described for Burg et al. (1995). It investigated intrasubregistry differences among 3,915 living members of the National Exposure Registry's Trichloroethylene Subregistry (4,041 total living members). The participants' mean age was 34 years (SD = 19.9 years), and included children in the registry. All registrants had been exposed to TCE through domestic use of contaminated well water. All were Caucasian. All registrants had been exposed to TCE though domestic use of contaminated well water; there were four exposure Subgroups, each divided into quartiles: (1) Maximum TCE measured in well water, exposure subgroups include 2−12 ppb; 12−60 ppb; 60−800 ppb; (2) Cumulative TCE exposure subgroups include <50 ppb, 50−500 ppb, 500−5,000 ppb, >5,000 ppb; (3) Cumulative chemical exposure subgroups include TCA, DCE, dichloroacetic acid (DCA), in conjunction with TCE, with the same exposure Categories as in # 2; and (4) Duration of exposure subgroups include <2 years, 2−5 years, 5−10 years, >10 years; 2,867 had TCE exposure of ≤50 ppb; 870 had TCE exposure of 51−500 ppb; 190 had TCE exposure of 501−5,000 ppb; 35 had TCE exposure >5,000 ppb. The lowest quartile was used as a control group. Interviews included occupational, environmental, demographic, and health information. A large number of health outcomes were analyzed, including speech impairment and hearing impairment. Statistical methods used include Logistic Regression and Odds Ratios. The primary purpose was to evaluate the rate of reporting health-outcome variables across exposure categories. The data were evaluated for an elevation of the risk estimates across the highest exposure categories or for a dose-response effect, while controlling for potential confounders. Estimated prevalence odds ratios for the health outcomes, adjusted for the potential confounders, were calculated by exponentiating the β-coefficients from the exposure variables in the regression equations. The standard error of the estimate was used to calculate 95% confidence intervals (CIs). The referent group used in the logistic regression models was the lowest exposure group. The results variables were modeled as dichotomous, binary dependent variables in the regression models. Nominal, independent variables were modeled, using dummy variables. The covariables used were sex, age, occupational exposure, education level, smoking history, and the sets of environmental subgroups. The analyses were restricted to persons 19 years of age or older when the variables of occupational history, smoking history, and education level were included. When the registrants were grouped by duration of exposure to TCE, a statistically significant association (adjusted for age and sex) between duration of exposure and reported hearing impairment was found. The prevalence odds ratios were 2.32 (95% Cl: 1.18, 4.56) (>2 to <5 years); 1.17 (95% Cl: 0.55, 2.49) (>5 to <10 years); and 2.46 (95% Cl = 1.30, 5.02) (>10 years). Higher rates of speech impairment (although not statistically significant) were associated with maximum and cumulative TCE exposure, and duration of exposure. The study reports dose-response relationships, but the effects are self reported, and exposure data are estimates. No information was reported on presence or absence of additional solvents in drinking water.

ATSDR (2003) conducted a follow-up study to the TCE subregistry findings (Burg et al., 1995, 1999) and focused on the subregistry children. Of the 390 subregistry children (≤10 years at time of original study), 116 agreed to participate. TCE exposure ranged from 0.4 to 5,000 ppb from the drinking water. The median TCE exposure for this subgroup was estimated to be 23 ppb per year of exposure. To further the hearing impairments reported in Burg et al. (1995, 1999), comprehensive auditory tests were conducted with the 116 children and compared to a control group of 182 children that was age-matched. The auditory tests consisted of a hearing screening (typanometry, pure tone and distortion product otoacoustic emissions [DPOAE]) and a more in-depth hearing evaluation for children that failed the initial screening. Ninety percent of the TCE-exposed children passed the typanometry and pure tone tests, and there were no significant differences between control and TCE-exposed groups. Central auditory processing tests were also conducted and consisted of a test for acoustic reflexes and a screening test for auditory processing disorders (SCAN). The acoustic reflex tested the ipsilateral and contralateral auditory pathway at 1,000 Hz for each ear. In this test, each subject hears the sound frequency and determines if the sound causes the stapedius muscle to tighten the stapes (normal reflex to noise). Approximately 20% of the children in the TCE subregistry and 5−7% in the controls exhibited an abnormal acoustic reflex, and this increased abnormality in the test was a significant effect ($p = 0.003$). No significant effects were noted in the SCAN tests. The authors concluded that the significant decrease in the acoustic reflex for the TCE subregistry children is reflective of potential abnormalities in the middle ear, which may reflect abnormalities in lower brainstem auditory pathway function. Lack of effects with the pure tone and typanometry tests suggests that the cochlea is not affected by TCE exposure.

Although auditory function was not directly measured, Rasmussen et al. (1993b) used a psychometric test to measure potential auditory effects of TCE exposure in an environmental study. Results from 96 workers exposed to TCE and other solvents were presented in this study. The workers were divided into three exposure groups: low, medium, and high. Details of the exposure groups and exposure levels are provided in Table 4-21 (under study description of Rasmussen et al., 1993b). Three auditory-containing tasks were included in this study, but only the acoustic motor function test could be used for evaluation of auditory function. In the acoustic motor function test, high and low frequency tones were generated and heard through a set of earphones. Each individual then had to imitate the tones by knocking on the table using the flat hand for a low frequency and using a fist for a high frequency. A maximal score of 8 could be achieved through this test. The tones were provided in either a set of 1 or 3 groups. In the one group acoustic motor function test, the average score for the low exposure group was 4.8 in comparison to 2.3 in the high exposure group. Similar decrements were noted in the 3 group acoustic motor function test. A significant association was reported for TCE exposure and performance on the one group acoustic motor function test ($p < 0.05$) after controlling for confounding variables.

D.1.3. Vestibular Effects

The data linking acute TCE exposure with transient impairment of vestibular function are quite strong based on human chamber studies, occupational exposure studies, and laboratory animal investigations. It is clear from the human literature that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature.

The earliest reports of neurological effects resulting from TCE exposures focused on subjective symptoms, such as headaches, dizziness, and nausea. These symptoms are subjective and self-reported, and, therefore, offer no quantitative measurement of cause and effect. However, there is little doubt that these effects can be caused by exposures to TCE, as they have been reported extensively in the literature, resulting from occupational exposures (Grandjean et al., 1955; Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith, 1970), environmental exposures (Hirsch et al., 1996), and in chamber studies (Stewart et al., 1970; Kylin et al., 1967). These studies are described below in more detail.

Grandjean et al. (1955) reported on 80 workers exposed to TCE from 10 different factories of the Swiss mechanical engineering industry. TCE air concentrations varied from 6−1,120 ppm (33−6,200 mg/m^3) depending on time of day and proximity to tanks, but mainly averaged between 20−40 ppm (100−200 mg/m^3). Urinalysis (TCA) varied from 30 mg/L to 300 mg/L. This study does not include an unexposed referent group, although prevalences of self-reported symptoms or neurological changes among the higher-exposure group are compared to the lower-exposure group. Workers were classified based on their exposures to TCE and there were significant differences ($p = 0.05$) in the incidence of neurological disorder between Groups I (10−20 ppm), II (20−40 ppm; 110−220 mg/m^3) and III (>40 ppm; 220 mg/m^3). Thirty-four percent of the workers had slight or moderate psycho-organic syndrome; 28% had neurological changes. Approximately 50% of the workers reported incidences of vertigo and 30% reported headaches (primarily an occasional and/or minimal disorder). Based on TCA eliminated in the urine, results show that subjective, vegetative, and neurological disorders were more frequent in Groups II (40−100 mg/L) and III (101−250 mg/L) than in Group I (10−39 mg/L). Statistics do support a dose-effect relationship between neurological effects and TCE exposure, but exposure data are questionable.

Liu et al. (1988) evaluated the effects of occupational TCE exposure on 103 factory workers in Northern China. The workers (79 men, 24 women) were exposed to TCE during vapor degreasing production or operation. An unexposed control group of 85 men and 26 women was included for comparison. Average TCE exposure was mostly at less than 50 ppm (275 mg/m^3). The concentration of breathing zone air during entire shift was measured by diffusive samplers placed on the chest of each worker. Subjects were divided into three exposure groups; 1−10 ppm (5.5−55 mg/m^3), 11−50 ppm (60−275 mg/m^3) and 51−100 ppm (280−550 mg/m^3). Results were based on a self-reported subjective symptom questionnaire. The frequency of subjective symptoms, such as nausea, drunken feeling, light-headedness, floating sensation, heavy feeling of the head, forgetfulness, tremors and/or cramps in extremities, body weight loss, changes in perspiration pattern, joint pain, and dry mouth (all ≥3 times more common in exposed workers); reported as 'prevalence of affirmative answers', was significantly greater in exposed workers than in unexposed ($p < 0.01$). *"Bloody strawberry jam-like feces"* was borderline significant in the exposed group and "*frequent flatus"* was statistically significant. Dose-response relationships were established (but not statistically significant) for symptoms. Most workers were exposed below 10 ppm, and some at 11−50 ppm. The differences in exposure intensity between men and women was of borderline significance ($0.05 < p < 0.10$). The study appears to be well done, although the self reporting of symptoms and the 'prevalence of affirmative answers' metric is not standard practice.

Rasmussen et al. (1986) conducted a cross-sectional study on 368 metal degreasers working in various factories in Denmark (industries not specified) with chlorinated solvents. The control group consisted of 94 randomly selected semiskilled metal workers from same area. The mean age was 37.7 (range: 17−65+). Neurological symptoms of the subjects were assessed by questionnaire. The workers were categorized into four groups as follows: (1)currently working with chlorinated solvents ($n = 171$; average duration: 7.3 years, 16.5 hours/week; 57% TCE and 37% 1,1,1-trichloroethane), (2) currently working with other solvents ($n = 131$;

petroleum, gasoline, toluene, xylene), (3) previously (1−5 years.) worked with chlorinated or other solvents ($n = 66$), (4) never worked with organic solvents ($n = 94$). A dose-response relationship was observed between exposure to chlorinated solvents and chronic neuropsychological symptoms including vestibular system effects such as dizziness ($p < 0.005$), and headache ($p < 0.01$). The authors indicated that TCE exposure resulted in the most overall symptoms. Significant associations were seen between previous exposure and consumption of alcohol with chronic neuropsychological symptoms. Results are confounded by exposures to additional solvents.

Smith (1970) conducted an occupational study on 130 workers (108 males, 22 females) exposed to TCE (industry not reported). The control group consisted of 63 unexposed men working at the same factories matched by age, marital status and other nonspecified criteria. A referent group was included and consisted of 112 men and women exposed to low concentration of lead and matched to the TCE exposed group in age and sex distribution. Seventy-three out of 130 workers (56.2%) reported dizziness and 23 workers reported having headaches (17.7%). The number of complaints reported by subjects was greater for those with 60 mg/L or greater TCA than for those with less than 60 mg/L TCA. There was no difference in the number of symptoms reported between those with shorter durations of exposure and those with longer durations of exposure. No statistics were reported.

Hirsh et al. (1996) evaluated the vestibular effects of an environmental exposure to TCE in Roscoe, IL residents. A medical questionnaire was mailed to 103 residents of Roscoe with 100% response. These 103 and an additional 15 residents, not previously surveyed, brought the subject population to 118 residents. During the course of testing, 12 subjects (young children and uncooperative patients) were excluded bringing the total number of subjects to 106 all of whom were in the process of taking legal action against the company whose industrial waste was assumed to be the source of the polluting TCE. This was a case series report with no controls. Random testing of the wells between 1983−84 revealed groundwater in wells to have levels of TCE between 0 to 2,441 ppb. The distance of residence from contaminated well was used to estimate exposure level. Sixty-six subjects (62%) complained of headaches at the time of evaluation. Diagnosis of TCE-induced cephalagia was considered credible for 57 patients (54%). Forty-seven of these had a family history of headaches. Retrospective TCE level of well water or well's distance from the industrial site analysis did not correlate with the occurrence of possibly-TCE induced headaches. This study shows a general association between headaches and exposure to TCE in drinking water wells. There were no statistics to support a dose-response relationship. All subjects were involved in litigation.

Stewart et al. (1970) evaluated vestibular effects in 13 subjects who were exposed to TCE vapor 100 ppm (550 mg/m^3) and 200 ppm (1,100 mg/m^3) for periods of 1 hour to a 5-day work week. Experiments 1−7 were for a duration of 7 hours with a mean TCE concentration of 198−200 ppm (1,090−1,100 mg/m^3). Experiments 8 and 9 exposed subjects to 190−202 ppm (1,045−1,110 mg/m^3) TCE for a duration of 3.5 and 1 hour, respectively. Experiment 10 exposed subjects to 100 ppm (550 mg/m^3) TCE for 4 hours. Experiments 2−6 were carried out with the same subjects over 5 consecutive days. Gas chromatography of expired air was measured. There were no self controls. Subjects reported symptoms of lightheadedness, headache, eye, nose, and throat irritation. Prominent fatigue and sleepiness by all were reported above 200 ppm (1,100 mg/m^3). There were no quantitative data or statistics presented regarding dose and effects of neurological symptoms.

Kylin et al. (1967) exposed 12 volunteers to 1,000 ppm (5,500 mg/m^3) TCE for 2 hours in a 1.5 × 2 × 2 meters chamber. Volunteers served as their own controls since 7 of the 12 were pretested prior to exposure and the remaining 5 were post-tested days after exposure. Subjects were tested for optokinetic nystagmus, which was recorded by electronystogmography, that is, “the potential difference produced by eye movements between electrodes placed in lateral angles between the eyes.” Venous blood was also taken from the volunteers to measure blood TCE levels during the vestibular task. The authors concluded that there was an overall reduction in the limit (“fusion limit”) to reach optokinetic nystagmus when individuals were exposed to TCE. Reduction of the “fusion limit” persisted for up to 2 hours after the TCE exposure was stopped and the blood TCE concentration was 0.2 mg/100 mL.

D.1.4. Visual Effects

Kilburn (2002a) conducted an environmental study on 236 people exposed to TCE in groundwater in Phoenix, AZ. Details of the TCE exposure and population are described earlier in Section D.1.1.1 (see Kilburn [2002a]). Among other neurological tests, the population and 161 nonexposed controls was tested for color discrimination using the desaturated Lanthony 15-hue test, which can detect subtle changes in color vision deficiencies. Color discrimination errors were significantly increased in the TCE exposed population ($p < 0.05$) with errors scores averaging 12.6 in the TCE exposed in comparison to 11.9 in the control group. This study shows statistically significant differences in visual response between exposed and nonexposed subjects exposed environmentally. Estimates of TCE concentrations in drinking water to individual subjects are lacking.

Reif et al. (2003) conducted a cross sectional environmental study on 143 residents of the Rocky Mountain Arsenal community of Denver whose water was contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986. The residents were divided into three groups based on TCE exposure with the lowest exposure group at <5 ppb, the medium exposure group at 5 to15 ppb and the high exposure group defined as

>15 ppb TCE. Visual performance was measured by two different contrast sensitivity tests (C and D) and the Benton visual retention test. In the two contrast sensitivity tests, there was a 20 to 22% decrease in performance between the low and high TCE exposure groups and approached statistical significance ($p = 0.06$ or 0.07). In the Benton visual retention test, which measures visual perception and visual memory, scores, dropped by 10% from the lowest exposure to the highest TCE exposure group and was not statistically significant. It should be noted that the residents were potentially exposed to multiple solvents including TCE and a nonexposed TCE group was not included in the study. Additionally, modeled exposure data are only a rough estimate of actual exposures, and possible misclassification bias associated with exposure estimation may limit the sensitivity of the study.

Rasmussen et al. (1993b) conducted a cross-sectional study on 96 metal workers, working in degreasing at various factories in Denmark (industries not specified) with chlorinated solvents. These subjects were identified from a larger cohort of 240 workers. Details of the exposure groups and TCE exposure levels are presented in Section D.1.1.1 (under Rasmussen et al., 1993c). Neuropsychological tests including the visual gestalts (test of visual perception and retention) and the stone pictures test (test of visual learning and retention) were administered to the metal workers. In the visual gestalts test, cards with a geometrical figure containing four items were presented and workers had to redraw the figure from memory immediately (learning phase) after presentation and after 1 hour (retention phase). In the learning phase, the figures were redrawn until the worker correctly drew the figure. The number of total errors significantly increased from the low group (3.4 errors) to the high exposure group (6.5 errors; $p = 0.01$) during the learning phase (immediate presentation). Similarly, during the retention phase of this task (measuring visual memory), errors significantly increased from an average of 3.2 in the low group to 5.9 in the high group ($p < 0.001$). In the stone pictures test, slides of 10 stones (different shapes and sizes) were shown and the workers had to identify the 10 stones out of a lineup of 25 stones. There were no significant changes in this task, but the errors increased from 4.6 in the low exposure group to 6.3 in the high exposure group during the learning phase of this task. Although this study identifies visual performance deficits, a control group (no TCE exposure) was not included in this study and the presented results may actually underestimate visual deficits from TCE exposure.

Troster and Ruff (1990) presented case studies conducted on two occupationally exposed workers to TCE and included a third case study on an individual exposed to 1,1,1-trichloroethane. Case #1 was exposed to TCE (concentration unknown) for 8 months and Case #2 was exposed to TCE over a 3-month period. Each patient was presented with a visual-spatial task (Ruff-Light Trail Learning test as referenced by the authors). Both of the individuals exposed to TCE were unable to complete the visual-spatial task and took the maximum number of trials (10) to attempt to complete the visual task. A control group of 30 individuals and the person exposed to 1,1,1-trichloroethane were able to complete this task accordingly. The lack of quantitative exposure data and a small sample size severely limits the study and does not allow for statistical comparisons.

Vernon and Ferguson (1969) exposed eight male volunteers (ages 21−30) to 0, 100, 300, and 1,000-ppm TCE for 2 hours. Each individual was exposed to all TCE concentrations and a span of at least 3 days was given between exposures. The volunteers were presented with six visuo-motor tests during the exposure sessions. When the individuals were exposed to 1,000-ppm TCE (5,500 mg/m^3), significant abnormalities were noted in depth perception as measured by the Howard-Dolman test ($p < 0.01$), but no effects on the flicker fusion frequency test (threshold frequency at which the individual sees a flicker as a single beam of light) or on the form perception illusion test (volunteers presented with an illusion diagram). This is one of the earliest chamber studies of TCE. This study included only healthy young males, is of a small size, limiting statistical power, and reports mixed results on visual testing following TCE exposure.

D.1.5. Cognition

There is a single environmental study in the literature that presents evidence of a negative impact on intelligence resulting from TCE exposure. Kilburn and Warshaw (1993—study details in Section D.1.1.1) evaluated the effects on cognition for 544 Arizona residents exposed to TCE in well-water. Subjects were recruited and categorized into three groups. Exposed Group 1 consisted of 196 family members with cancer or birth defects. Exposed Group 2 consisted of 178 individuals from families without cancer or birth defects; and exposed Group 3 included 170 parents whose children had birth defects and rheumatic disorders. Sixty-eight referents were used as a comparison group for the clinical memory tests. Several cognitive tests were administered to these residents in order to test memory recall skills and determine if TCE exposure resulted in memory impairment. Working or short-term memory skills were tested by asking each individual to recall two stories immediately after presentation (verbal recall) and also draw three diagrams immediately after seeing the figures (visual recall). Additionally, a digit span test where increasing numbers of digits were presented and then the subject had to recall the digits was conducted to the extent of the short-term memory. Exposed subjects had lower intelligence scores and there were significant impairments in verbal recall ($p = 0.001$), visual recall ($p = 0.03$) and with the digit span test ($p = 0.07$). Significant impairment in short-term memory as measured by three different cognitive test was correlated with TCE exposure. Lower intelligence scores ($p = 0.0001$) as measured by the Culture Fair IQ test may be a possible confounder in these findings. Additionally, the large range of TCE concentrations

(6−500 ppb) and exposure durations (1 to 25 years) and overall poor exposure characterization precludes a no-observed-adverse-effect level (NOAEL)/lowest-observed-adverse-effect level (LOAEL) from being estimated from this study on cognitive function.

Rasmussen et al. (1993a, b) and Troster and Ruff (1990) present results of positive findings in occupational studies for cognitive effects of TCE. Rasmussen et al. (1993a) reported an historical cohort study conducted on 96 metal degreasers, identified 2 years previously and were selected from a population of 240 workers from 72 factories in Denmark. They reported psychoorganic syndrome, a mild syndrome of dementia characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative, was increased in the three exposure groups. The medium and high exposure groups were compared with the low exposure group. Neuropsychological tests included WAIS (original version, Vocabulary, Digit Symbol, Digit Span), Simple Reaction Time, Acoustic-motor function (Luria), Discriminatory attention (Luria), Sentence Repetition, Paced Auditory Serial Addition Test (PASAT), Text Repetition, Rey's Auditory Verbal Learning, Visual Gestalts, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, and Mira. The prevalence of psychoorganic syndrome was 10.5% in low exposure group; 38.9% in medium exposure group; 63.4% in high exposure group. (x^2 trend analysis: low vs. medium exposure $x^2 = 11.0$, p value <0.001; low vs. high exposure $x^2 = 19.6$, p-value <0.001.) Psychoorganic syndrome increased with age ($p < 0.01$). Age was strongly correlated with exposure.

Rasmussen et al. (1993b) used a series of cognitive tests to measure effects of occupational TCE exposure. Short-term memory and retention following an latency period of one hour was evaluated in several tests including a verbal recall (auditory verbal learning test), visual gestalts, visual recall (stone pictures), and the digit span test. Significant cognitive performance decreases were noted in both short-term memory and memory retention. In the verbal recall test immediate memory and learning were significantly decreased ($p = 0.03$ and 0.04, respectively). No significant effects were noted for retention following a one hour latency period was noted. Significant increases in errors were noted in both the learning ($p = 0.01$) and memory ($p < 0.001$) phases for the visual gestalts test. No significant effects were found in the visual recall test in either the learning or memory phases or in the digit span test. As a result, there were some cognitive deficits noted in TCE-exposed individuals as measured through neuropsychological tests.

Troster and Ruff (1990) provides additional supporting evidence in an occupational study for cognitive impairment, although the results reported in a qualitative fashion are limited in their validity. In the two case studies that were exposed to TCE, there were decrements (no statistical analysis performed) in cognitive performance as measured in verbal and visual recall tests that were conducted immediately after presentation (learning phase) and one hour after original presentation (retention/memory phase).

Triebig et al. (1977b) presents findings of no impairment of cognitive ability resulting from TCE exposure in an occupational setting. This study was conducted on 8 subjects occupationally exposed to TCE. Subjects were 7 men and 1 woman with an age range from 23−38 years. Measured TCE in air averaged 50 ppm (260 mg/m^3). Length of occupational exposure was not reported. There was no control group. Results were compared after exposure periods, and compared to results obtained after periods removed from exposure. TCA and TCE metabolites in urine and blood were measured. The testing consisted of the Syndrome Short Test, which consists of nine subtests through which amnesic and simple perceptive and cognitive functional deficits are detected; the "Attention Load Test" or "d2 Test" from Brickenkamp is a procedure that measures attention, concentration, and stamina. Number recall test, letter recall test, the "Letter Reading Test," "Word Reading Test." Data were assessed using Wilcoxon and Willcox nonparametric tests. Due to the small sample size a significance level of 1% was used. The concentrations of TCE, trichloroethanol, and TCA in the blood and total TCE and total TCA elimination in the urine were used to assess exposure in each subject. The mean values observed were 330 mg trichloroethanol and 319 mg TCA/g creatinine, respectively, at the end of a work shift. The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period. The small sample size may limit the sensitivity of the study.

Salvini et al. (1971), Gamberale et al. (1976), and Stewart et al. (1970) reported positive findings for the impairment of cognitive function following TCE exposures in chamber studies. Salvini et al. (1971) reported a controlled exposure study conducted on six male university students. TCE concentration was 110 ppm (550 mg/m^3) for 4-hour intervals, twice per day. Each subject was examined on two different days, once under TCE exposure, and once as self controls, with no exposure. Two sets of tests were performed for each subject corresponding to exposure and control conditions. The test battery included a perception test with tachistoscopic presentation, the Wechsler memory scale test, a complex reaction time test, and a manual dexterity test. Statistically significant results were observed for perception tests learning ($p < 0.001$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$); and CRT learning ($p < 0.01$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$). This is controlled exposure study with measured dose (110 ppm; 600 mg/m^3) and clear, statistically significant impact on neurological functional domains. However, it only assesses acute exposures.

Gamberale et al. (1976) reported a controlled exposure study conducted on 15 healthy men aged 20−31 yrs old, employed by the Department of Occupational Medicine in Stockholm, Sweden. Controls were within subjects (15 self-controls), described above. Test used included reaction time (RT) Addition and short term memory using an electronic panel. Subjects also assessed their own conditions on a 7-point scale. Researchers used a repeated measures analysis of variance (ANOVA) for the 4 performance tests based on a 3×3 Latin square design. In the short-term memory test (version of the digit span test), a series of numbers lasting for one second was presented to the subject. The volunteer then had to reproduce the numerical sequence after a latency period (not specified). No significant effect on the short-term memory test was observed with TCE exposure in comparison to air exposure. Potential confounders from this study include repetition of the same task for all exposure conditions, volunteers served as their own controls, and TCE exposure preceded air exposure in two of the three exposure experimental designs. This is a well controlled study of short term exposures with measured TCE concentrations and significant response observed for cognitive impairment.

Additional qualitative support for cognitive impairment is provided by Stewart et al. (1970). This was a controlled exposure study conducted on 13 subjects in 10 experiments, which consisted of ten chamber exposures to TCE vapor of 100 ppm (550 mg/m^3) and 200 ppm (1,100 mg/m^3) for periods of 1 hour to a 5-day work week. Experiments 1−7 were for 7 hours with a mean TCE concentration of 198−200 ppm (1,090−1,100 mg/m^3). Experiments 8 and 9 exposed subjects to 190−202 ppm (1,045−1,110 mg/m^3) TCE for a duration of 3.5 and 1 hour, respectively. Experiment 10 exposed subjects to 100 ppm (550 mg/m^3) TCE for 4 hours. Experiments 2−6 were carried out with the same subjects over 5 consecutive days. Gas chromatography of expired air was measured. There were no self controls. All had normal neurological tests during exposure, but 50% reported greater mental effort was required to perform a normal modified Romberg test on more than one occasion. There were no quantitative data or statistics presented regarding dose and effects of neurological symptoms.

Two chamber studies conducted by Triebig et al. (1976, 1977a) report no impact of TCE exposure on cognitive function. Triebig et al. (1976) was a controlled exposure study conducted on 7 healthy male and female students (4 females, 3 males) exposed for 6 hours/day for 5 days to 100 ppm (550 mg/m^3 TCE). The control group was 7 healthy students (4 females, 3 males) exposed to hair care products. This was assumed as a zero exposure, but details of chemical composition were not provided. Biochemical and psychological testing was conducted at the beginning and end of each day. Biochemical tests included TCE, TCA, and trichloroethanol in blood. Psychological tests included the d2 test, which was an attention load test; the short test (as characterized in the translated version of Treibig, 1976) is used to record patient performance with respect to memory and attention; daily Fluctuation Questionnaire measured the difference between mental states at the start of exposure and after the end of exposure is recorded; The MWT-A is a repeatable short intelligence test; Culture Fair Intelligence Test (CFT-3) is a nonverbal intelligence test that records the rather "fluid" part of intelligence, that is, finding solution strategies; Erlanger Depression Scale. Results were not randomly distributed. The median was used to describe the mean value. Regression analyses were conducted. In this study the TCE concentrations in blood reported ranged from 4 to 14 μg/mL. A range of 20 to 60 μg/mL was obtained for TCA in the blood. There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. The biochemical data did demonstrate subjects' exposures. This is a well controlled study with excellent exposure data, although the small sample size may have limited sensitivity.

Triebig et al. (1977a) is an additional report on the seven exposed subjects and seven controls evaluated in Triebig et al. (1976). Additional psychological testing was reported. The testing included the Syndrome Short Test, which consists of nine subtests, described above. Statistics were conducted using Whitney Mann. Results indicated the anxiety values of the placebo random sample group dropped significantly more during the course of testing ($p < 0.05$) than those of the active random sample group. No significantly different changes were obtained with any of the other variables. Both these studies were well controlled with excellent exposure data, which may provide some good data for establishing a short term NOAEL. The small sample size may have limited the sensitivity of the study.

Additional reports on the impairment of memory function as a result of TCE exposures have been reported, and provide additional evidence of cognitive impairment. The studies by Chalupa et al. (1960), Rasmussen et al. (1986, 1993b), and Troster and Ruff (1990) report impairment of memory resulting from occupational exposures to TCE. Kilburn and Warshaw (1993) and Kilburn (2002a) report impairment of memory following environmental exposures to TCE. Salvini et al. (1971) reports impairment of memory in a chamber study, although Triebig et al. (1976) reports no impact on memory following TCE exposure in a chamber study.

D.1.6. Psychomotor Effects

There is evidence in the literature that TCE can have adverse psychomotor effects in humans. The effects of TCE exposure on psychomotor response have been studied primarily as the impact on RTs, which provide a quantitative measure of the impact TCE exposure has on motor skills. Studies on motor dyscoordination resulting from TCE exposure are more subjective, but provide additional evidence that TCE may cause adverse psychomotor effects. These studies are described below.

D.1.6.1. *Reaction Time*

There are several reports in the literature that report an increase in reaction times following exposures to TCE. The best evidence for TCE exposures causing an increase in choice reaction times comes from environmental studies by Kilburn (2002a), Kilburn and Warshaw

(1993), Reif et al. (2003), and Kilburn and Thornton (1996), which were all conducted on populations which were exposed to TCE through groundwater contaminated as the result of environmental spills. Kilburn (2002a—study details described in Section D.1.1) evaluated reaction times in a Phoenix, Arizona population exposed to TCE through groundwater. Volunteers were tested for response rates in the simple reaction time (SRT) and 2 choice reaction time (CRT) tests. Various descriptive statistics were used, as well as analysis of covariance (ANCOVA) and a step-wise adjustment of demographics. The principal comparison, between the 236 exposed persons and the 161 unexposed regional controls, revealed significant differences ($p < 0.05$) indicating that SRTs and CRTs were delayed. Balance was also abnormal with excessive sway speed (eyes closed), but this was not true when both eyes were open. This study shows statistically significant differences in psychomotor responses between exposed and nonexposed subjects exposed environmentally. However, it is limited by poor exposure characterization.

Kilburn and Warshaw (1993; study details described in Section D.1.1.1) evaluated reaction times in 170 Arizona residents exposed to TCE in well water. A referent group of 68 people was used for comparison. TCE concentration was from 6 to 500 ppb and exposure ranged from 1 to 25 years. SRT was determined by presenting the subject a letter on a computer screen and measuring the time (in milliseconds [msec]) it took for the person to type that letter. SRT significantly increased from 281 ± 55 msec to 348 ± 96 msec in TCE-exposed individuals ($p < 0.0001$). Similar increases were reported for CRT where subjects were presented with two different letters and required to make a decision as to which letter key to press. CRT of the exposed subjects was 93 msec longer in the third trial ($p < 0.0001$) than referents. It was also longer in all trials, and remained significantly different after age adjustment. This study shows statistically significant differences for neurological test results between subjects environmentally exposed and nonexposed to TCE, but is limited by poor exposure data on individual subjects given the ecological design of this study. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Kilburn and Thornton (1996) conducted an environmental study that attempts to use reference values from two control groups in assessing neurological responses for chemically exposed subjects using neurophysiological and neuropsychological testing on three groups. Group A included randomly selected registered voters from Arizona and Louisiana with no exposure to TCE: $n = 264$ unexposed volunteers aged 18−83. Group B included volunteers from California $n = 29$ (17 males and 12 females) that were used to validate the equations; Group C included those exposed to TCE and other chemicals residentially for 5 years or more $n = 237$. Group (A), was used to develop the regression equations for SRT and choice reaction time (CRT). A similarly selected comparison group B was used to validate the equations. Group C, the exposed population, was submitted to SRT and CRT tests ($n = 237$) and compared to the control groups. All subjects were screened by a questionnaire. Reaction speeds were measured using a timed computer visual-stimulus generator. No exposure data were presented. The Box-Cox transformation was used for dependent variables and independent variables. They evaluated graphical methods to study residual plots. Cook's distance statistic was used as a measure of influence to exclude outliers with undue influence and none of the data were excluded. Lack-of-fit test was performed on Final model and F statistic was used to compare estimated error to lack-of-fit component of the model's residual sum of squared error. Final models were validated using group B data and paired t-test to compare observed values for SRT and CRT. F statistic was used to test the hypothesis that parameter estimates obtained with group B were equal to those of Group A, the model. The results are as follows: Group A: SRT = 282 ms; CRT = 532 ms. Group B: SRT = 269 ms; CRT = 531 ms. Group C: SRT = 334 ms; CRT = 619 ms. TCE exposure produced a step increase in reaction times (SRT and CRT). The coefficients from Group A were valid for group B. The predicted value for SRT and for CRT, plus 1.5 SDs selected 8% of the model group as abnormal. The model produced consistent measurement ranges with small numerical variation. This study is limited by lack of any exposure data, and does not provide statistics to demonstrate dose-response effects.

Kilburn (2002a) conducted an environmental study on 236 residents chronically exposed to TCE-associated solvents in the groundwater resulting from a spill from a microchip plant in Phoenix, AZ. Details of the TCE exposure and population are described earlier in Section D.1.1.1 (see Kilburn [2002a]). The principal comparison, between the 236 exposed persons and the 161 unexposed regional controls, revealed significant differences indicating that SRTs and choice reaction times (CRTs) were increased. SRTs significantly increased from 283 ± 63 msec in controls to 334 ± 118 msec in TCE exposed individuals ($p < 0.0001$). Similarly, CRTs also increased from 510 ± 87 msec to 619 ± 153 msec with exposure to TCE ($p < 0.0001$). This study shows statistically significant differences in psychomotor responses as measured by reaction times between TCE-exposed and nonexposed subjects. Estimates of TCE concentrations in drinking water to individual subjects were not reported in the paper. Since the TCE exposure ranged from 0.2 to over 10,000 ppb in well water, it is not possible to determine a NOAEL for increased reaction times through this study. Additionally, litigation is suggested and may introduce a bias, particularly if no validity tests were used.

Reif et al. (2003) conducted a cross sectional study on 143 residents of the Rocky Mountain Arsenal (RMA) community of Denver exposed environmentally to drinking water contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986. The referent group was at the lowest estimated exposure concentration (<5 ppb). The socioeconomic profile of the participants closely resembled those of the community in general.

"A total of 3393 persons was identified through the census, from which an age- and gender-stratified sample of 1267 eligible individuals who had lived at their current residence for at least 2 years was drawn. Random selection was then used to identify 585 persons from within the age–gender strata, of whom 472 persons aged 2–86 provided samples for biomonitoring. Neurobehavioral testing was conducted on 204 adults who lived in the RMA exposure area for a minimum of 2 years. Among the 204 persons who were tested, 184 (90.2%) lived within the boundaries of the LWD and were originally considered eligible for the current analysis. Therefore, participants who reported moving into the LWD after 1985 were excluded from the total of 184, leaving 143 persons available for study." An elaborate hydraulic simulation model (not validated) was used in conjunction with a geographic information system (GIS) to model estimates of residential exposures to TCE. The TCE concentration measured in community wells exceeded the MCL of 5 ppb in 80% of cases. Approximately 14% of measured values exceeded 15 ppb. Measured values were used to model actual exposure estimates based on distance of residences from sampled wells. The estimated exposure for the high exposure group was >15 ppb; the estimate for the low exposure referent group was <5 ppb. The medium exposure group was estimated at exposures $5 < x < 15$ ppb TCE. The test battery consisted of the Neurobehavioral Core Test Battery (NCTB), which consists of 7 neurobehavioral tests including simple reaction time. Results were assessed using the Multivariate Model. Results were statistically significant ($p < 0.04$) for the simple reaction time tests. The results are confounded by exposures to additional solvents and modeled exposure data, which while highly technical, are still only a rough estimate of actual exposures, and may limit the sensitivity of the study.

Gamberale et al. (1976) conducted a controlled exposure (chamber) study on 15 healthy men aged 20−31 yrs old, employed by the Department of Occupational Medicine in Stockholm, Sweden. Controls were within subjects (15 self-controls). Subjects were exposed to TCE for 70 minutes via a breathing valve to 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), and to ordinary atmospheric air (0 ppm). Sequence was counterbalanced between the 3 groups, days, and exposure levels. Concentration was measured with a gas chromatographic technique every third minute for the first 50 minutes, then between tests thereafter. Test used were RT addition, simple RT, choice RT and short term memory using an electronic panel. Subjects also assessed their own conditions on a 7 point scale. The researchers performed Friedman two-way analysis by ranks to evaluate differences between the 3 conditions. The results were nonsignificant when tested individually, but significant when tested on the basis of six variables. Nearly half of the subjects could distinguish exposure/nonexposure. Researchers performed ANOVA for the four performance tests based on a 3 × 3 Latin square design with repeated measures. In the RT-Addition test the level of performance varied significantly between the different exposure conditions ($F[2.24] = 4.35$; $p < 0.05$) and between successive measurement occasions

($F[2.24] = 19.25$; $p < 0.001$). The level of performance declined with increased exposure to TCE, whereas repetition of the testing led to a pronounced improvement in performance as a result of the training effect. No significant interaction effects were observed between exposure to TCE and training. This is a good study of short term exposures with measured TCE concentrations and significant response observed for reaction time.

Gun et al. (1978) conducted an occupational study on 8 TCE-exposed workers who operated degreasing baths in two different plants. Four female workers were exposed to TCE only in one plant and four female workers were exposed to TCE and nonhalogenated hydrocarbon solvents in the second plant. The control group ($n = 8$) consisted of 4 female workers from each plant who did not work near TCE. Each worker worked 2 separate 4-hour shifts daily, with one shift exposed to TCE and the second 4-hour shift not exposed. Personal air samples were taken continuously over separate 10-minute sessions. Readings were taken every 30 seconds. Eight-choice reaction times were carried out in four sessions; at the beginning and end of each exposure to TCE or TCE + solvents; a total of 40 reaction time trials were completed. TCE concentrations in the TCE only plant 1 (148−418 ppm [800−2,300 mg/m^3]) were higher than in the TCE + solvent plant 2 (3−87 ppm (16−480 mg/m^3). Changes in choice reaction times (CRT) were compared to level of exposure. The TCE only group showed a mean increase in reaction time, with a probable cumulative effect. In the TCE + solvent group, mean reaction time shortened in Session 2, then increased to be greater than at the start. Both control groups showed a shortening in mean choice reaction time in Session 2, which was sustained in Sessions 3 and 4 consistent with a practice effect. This is a study with well-defined exposures and reports of cause and effect (TCE exposure on reaction time); however, no statistics were presented to support the conclusions or the significance of the findings, and the small sample size is a limitation of the study.

D.1.6.2. *Muscular Dyscoordination*

Effects on motor dyscoordination resulting from TCE exposure have been reported in the literature. These impacts are subjective, but may provide additional evidence that TCE can cause adverse psychomotor effects. There are three reports summarized below which suggest that muscular dyscoordination resulted from TCE exposure, although all three have significant limitations due to confounding factors. Rasmussen et al. (1993c) presented findings on muscular dyscoordination as it relates to TCE exposure. This was a historical cohort study conducted on 96 metal degreasers, identified 2 years previously. Subjects were selected from a population of 240 workers from 72 factories in Denmark. Although the papers report a population of 99 participants, tabulated results were presented for a total of only 96. No explanation was provided for this discrepancy. These workers had chronic exposure to fluorocarbon (CFC 113)

($n = 25$) and mostly TCE ($n = 70$; average duration: 7.1 years.). There were no external controls. The range of working full-time degreasing was 1 month to 36 years. Researchers collected data regarding the workers' occupational history, blood and urine tests, as well as biological monitoring for TCE and TCE metabolites. A chronic exposure index (CEI) was calculated based on number of hours per week worked with solvents multiplied by years of exposure multiplied by 45 weeks per year. No TCE air concentrations were reported. Participants were categorized into three groups: (1) "Low exposure:" $n = 19$, average full-time exposure = 0.5 years. (2) "Medium exposure:" $n = 36$, average full-time exposure = 2.1 years. (3) "High exposure:" $n = 41$, average full-time exposure = 11 years. The mean TCA level in the "high" exposure group was 7.7 mg/L (max = 26.1 mg/L). Time-weighted average (TWA) measurements of CFC 113 levels were 260−420 ppm (U.S. and Danish TLV was 500 ppm). A significant trend of dyscoordination from low to high solvent exposure was observed ($p = 0.003$). This study provides evidence of causality for muscular dyscoordination resulting from exposure to TCE, but no measured exposure data were reported.

Additional evidence of the psychomotor effects caused by exposure to TCE are presented in Gash et al. (2007) and Troster and Ruff (1990). There are, however, significant limitations with each of these studies. In Gash et al. (2007), the researchers evaluated the clinical features of 1 Parkinson's disease (PD) patient, identified in a Phase 1 clinical trial study, index case, and an additional 29 coworkers of the patient, all with chronic occupational exposures to TCE. An additional 2 subjects with Parkinson's Disease were included, making the total of 3 Parkinson's disease patients, and 27 non-Parkinson's coworkers making up the study population. Coworkers for the study were identified using a mailed questionnaire to 134 former coworkers. No details are provided in the paper on selection criteria for the 134 former coworkers. Of the 134 former workers sent questionnaires, 65 responded. Twenty-one self-reported no symptoms, 23 endorsed 1−2 symptoms, and 21 endorsed 3 or more signs of Parkinsonism. Fourteen of the 21 with 3 or more signs and 13 of the 21 without any signs agreed to a clinical exam; this group comprises the 27 additional workers examined for Parkinsonian symptoms. No details were provided on nonresponders. All subjects were involved in degreasing with long-term chronic exposure to TCE through inhalation and dermal exposure (14 symptomatic: age range = 31−66, duration of employment range: 11−35 yrs) (13 asymptomatic: age range = 46−63, duration of employment range: 8−33 yrs). The data were compared between groups and with data from 110 age- matched controls. Exposure to TCE is self-reported and based on job proximity to degreasing operations. The paper lacks any description of degreasing processes including TCE usage and quantity. Mapping of work areas indicated that workers with PD worked next to the TCE container, and all symptomatic workers worked close to the TCE container. Subjects underwent a general physical exam, neurological exam and Unified Parkinson's Disease Rating Scale (UPDRS), timed motor tests, occupational history survey, and mitochondrial neurotoxicity. ANOVA analysis was conducted, comparing symptomatic versus nonsymptomatic workers, and comparing symptomatic workers to age-matched nonexposed controls. No description of the control population ($n = 110$), nor how data were obtained for this group, was presented. The symptomatic non-Parkinson's group was significantly slower in fine motor hand movements than age-matched nonsymptomatic group ($p < 0.001$). The symptomatic group was significantly slower ($p < 0.0001$) than age-matched unexposed controls as measured in fine motor hand movements on the Movement Analysis Panel. All symptomatic workers had positive responses to 1 or more questions on UPDRS Part II (diminished activities of daily life), and/or deteriorization of motor functions on Part III. The fine motor hand movement times of the asymptomatic TCE-exposed group were significantly slower ($p < 0.0001$) than age-matched nonexposed controls. Also, in TCE-exposed individuals, the asymptomatic group's fine motor hand movements were slightly faster ($p < 0.01$) than those of the symptomatic group. One symptomatic worker had been tested 1 year prior and his UPDRS score had progressed from 9 to 23. Exposures are based on self-reported information, and no information on the control group is presented. One of the PD patients predeceased the study and had a family history of PD.

Troster and Ruff (1990) reported a case study conducted on two occupationally exposed workers to TCE. Patients were exposed to low levels of TCE. There were 2 groups of $n = 30$ matched controls (all age and education matched) whose results were compared to the performance of the exposed subjects. Exposure was described as "Unknown amount of TCE for 8 months." Assessment consisted of the San Diego Neuropsychological Test Battery (SDNTB) and "1 or more of" Thematoc Apperception Test (TAT), Minnesota Multiphasic Personal Inventory (MMPI), and Rorschach. Medical examinations were conducted, including neurological, CT scan, and/or chemo-pathological tests, and occupational history was taken, but not described. There were no statistical results reported. Results were reported for each test, but no tests of significance were included, therefore, the authors presented their conclusions for each "case" in qualitative terms, as such: Case 1: Intelligence "deemed" to drop from premorbid function at 1 year 10 months after exposure. Impaired functions improved for all but reading comprehension, visuospatial learning and categorization (abstraction). Case 2: Mild deficits in motor speed, but symptoms subsided after removal from exposure.

D.1.7. Summary Tables

The following Tables (D-1 through D-3) provide a detailed summary of all the neurological studies conducted with TCE in humans. Tables D-1 and D-2 summarize each individual human study where there was TCE exposure. Table D-1 consists of studies where humans were primarily or soley exposed to TCE. Table D-2 contains human studies where there was a mixed solvent exposure and TCE was one of the solvents in the mixture. For each study summary, the study population, exposure assessment, methods, statistics, and results are provided. Table D-3 indicates the neurological domains that were tested from selected references (primarily from Table D-1).

Table D-1. Epidemiological studies: Neurological effects of trichloroethylene

<table>
<tr><th>Reference</th><th>Study population</th><th>Exposure assessment and biomarkers</th><th>Tests used</th><th>Statistics</th><th>Results</th></tr>
<tr>
<td>Barret et al., 1984</td>
<td>188 workers exposed to TCE occupationally from small and large factories in France (type of factories not disclosed); average age = 41; 6 yrs average exposure time.

The workers were divided into high and low exposure groups for both TCE and urinary TCA. No control group was mentioned.</td>
<td>Review of medical records and analysis of TCE atmospheric levels (detector tubes) and level of urinary metabolites measurement (TCA). TCE exposure groups included high exposure group (>150 ppm; n = 54) and low exposure group <150 ppm; n = 134). Personal factors including age, tobacco use, and alcohol intake were also analyzed; Exposure duration = 7 h/d for 7 yrs; no mention was made regarding whether or not the examiners were blind to the subjects' exposure status.</td>
<td>Complete physical examination including testing visual performance (acuity and color perception), evoked trigeminal potential latencies and audiometry, facial sensitivity, reflexes, and motoricity of the masseter muscles.</td>
<td>X^2 examined distribution of the different groups for comparing high and low exposed workers, one way analysis of variance, Mann Whitney U and t-test for analyzing personal factors.</td>
<td>Symptoms for which TCE role is statistically significant include the following: Trigeminal nerve impairment was reported in 22.2% (n = 12) of workers in the high-exposure group for TCE, 7.4% (n = 10) in the low-exposure group for TCE, 24.4% (n = 10) in the high-exposure group for TCA and 8.2% (n = 12) in the low-exposure group for TCA.

<table>
<tr><th>TCE Results</th><th>High dose%</th><th>Low dose%</th><th>p</th></tr>
<tr><td>Trigeminal nerve</td><td>22.2</td><td>7.4</td><td><0.01</td></tr>
<tr><td>Impairment asthenia</td><td>18.5</td><td>4.5</td><td><0.01</td></tr>
<tr><td>Optic nerve impairment</td><td>14.8</td><td>0.75</td><td><0.001</td></tr>
<tr><td>Headache</td><td>20.3</td><td>19.4</td><td>NS</td></tr>
<tr><td>Dizziness</td><td>13</td><td>4.5</td><td>$0.05 < p < 0.06$</td></tr>
</table>

Symptoms for which TCE role is possible, but not statistically significant = deafness, nystagmus, GI symptoms, morning cough, change in tumor, eczema, palpitations, conjunctivitis. Symptoms for which there is a synergistic toxic role for TCE and alcohol ($p < 0.05$) = liver impairment and degreaser flush. Trigeminal sensory evoked potentials are suggested as a good screening test.</td>
</tr>
</table>

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Barret et al., 1987	104 occupationally exposed workers highly exposed to TCE during work as degreaser machine operators in France. Controls: 52 healthy, nonexposed controls of various ages who were free from neurological problems.	Urinary analysis determined TCE and TCA rates. The average of the last 5 measurements were considered indicative of the average level of past exposure. Mean exposure 8.2 yrs, average daily exposure 7 hrs/d. Mean age 41.6 yrs.	Evoked trigeminal potentials were studied while eyes closed and fully relaxed. Also, physical exams with emphasis on nervous system, a clinical study of facial sensitivity, and of the reflexes depending on the trigeminal nerve were systematically performed. Normal latency and amplitude values for TSEP obtained from data from control population. Normal response characterized from 4 main peaks, alternating from negative to positive, respective latency of 12.8 ms (SD = 0.6), 19.5 ms (SD = 1.3), 27.6 ms (SD = 1.6), and 36.8 ms (SD = 2.2), mean amplitude of response is 2.5 μv (SD = 0.5 μv). Pathological responses were results 2 ½ SDs over the normal value.	Student's t-test and one-way ANOVA used as well as nonparametric tests Mann-Whitney U test and Kruskal-Wallis test. Also decision matrix and the analysis of the receiver operating curve to appreciate the accuracy of the TSEP method. The distribution of the different populations was compared by a chi square test.	Dizziness (71.4%), headache (55.1%), asthenia (46.9%), insomnia (24.4%), mood perturbation (20.4%), and sexual problems (12.2%) were found. Symptomatic patients had significantly longer exposure periods and were older than asymptomatic patients. 17.3% of patients had trigeminal nerve symptoms. Bilateral hypoesthesia with reflex alterations in 9 cases. Hypoesthesia was global and predominant in the mandibular and maxillary nerve areas. Several reflex abolitions were found without facial palsy and without convincing hypoesthesia in 9 cases. Corneal reflexes were bilaterally abolished in 5 cases as were naso-palpebral reflexes in 6 cases; length of exposure positively correlated with functional manifestations ($p < 0.01$); correlation between symptoms and exposure levels were nonsignificant; 40 (38.4%) subjects had pathological response to TSEP with increased latencies, amplitude or both; of these 28 had normal clinical trigeminal exam and 12 had abnormal exam; TSEP was positively correlated with length of exposure ($p < 0.01$); and with age ($p < 0.05$), but not with exposure concentration; trigeminal nerve symptoms ($n = 18$) were positively correlated with older age ($p < 0.001$).

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Barret et al., 1982	Eleven workers with chronic TCE exposure; 9 were suffering effects of solvent intoxication; 2 were work place controls. Control group was 20 unexposed subjects of all ages.	Selected following clinical evaluations of their facial sensitivity and trigeminal nerve reflexes; exposures verified by urinalysis. Presence of TCE and TCA found. (Exposure rates not reported).	Somatosensory evoked potential (SEP) following stimulation of the trigeminal nerve through the lip alternating right and left by a bipolar surface electrode utilizing voltage, usually 75 to 80 V, just below what is necessary to stimulate the orbicularis oris muscle. Duration was approx. 0.05 ms stimulated 500 times (2×/sec).	SEP recordings illustrated from trigeminal nerve graphs.	3 pathological abnormalities present in exposed (TCE intoxicated) workers: (1) in 8 workers higher voltage required to obtain normal response, (2) excessive delay in response observed twice, (3) excessive graph amplitude noted in 3 cases. One subject exhibited all 3 abnormalities. Correlation was reported between clinical observation and test results. Most severe SEP alternations observed in subjects with the longest exposure to TCE (although exposure levels or exposure durations are not reported). No statistics presented.
Burg et al., 1995	From an NHIS TCE subregistry of 4,281 (4,041 living and 240 deceased) residents environmentally exposed to TCE via well water in Indiana, Illinois, and Michigan; compared to NHIS registrants.	Morbidity baseline data were examined from the TCE Subregistry from the NER developed by the ATSDR; were interviewed in the NHIS.	Self report via face-to-face interviews—25 questions about health conditions; were compared to data from the entire NHIS population; neurological endpoints were hearing and speech impairments.	Poisson Regression analysis model used for registrants 19 and older. Maximum likelihood estimation and likelihood ratio statistics and Wald CI; TCE subregistry population was compared to larger NHIS registry population.	Speech impairments showed statistically significant variability in age-specific risk ratios with increased reporting for children ≤9 yrs (RR: 2.45, 99% CI: 1.31, 4.58) and for registrants ≥35 yrs (data broken down by 10-yr ranges). Analyses suggest a statistically significant increase in reported hearing impairments for children ≤9 yrs (RR: 2.13, 99% CI: 1.12, 4.06). It was lower for children 10−17 yrs (RR: 1.12, 99% CI: 0.52, 2.44) and ≤0.32 for all other age groups.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Burg and Gist, 1999	4,041 living members of the National Exposure Registry's Trichloroethylene Subregistry; 97% white; mean age 34 yrs (SD = 19.9 yrs.); divided in 4 groups based on type and duration of exposure; analysis reported only for 3,915 white registrants; lowest quartile used as control group.	All registrants exposed to TCE though domestic use of contaminated well water; 4 exposure Subgroups, each divided into quartiles: (1) Maximum TCE measured in well water, exposure subgroups: 2−12 ppb; 12−60 ppb; 60−800 ppb; (2) Cumulative TCE exposure subgroups: <50 ppb, 50−500 ppb, 500−5,000 ppb, >5,000 ppb; (3) Cumulative chemical exposure subgroups: include TCA, DCE, DCA, in conjunction with TCE, with the same exposure Categories as in # 2; (4) Duration of exposure subgroups: <2 yrs, 2−5 yrs, 5−10 yrs., >10 yrs.; 2,867 had TCE exposure of ≤50 ppb; 870 had TCE exposure of 51−500 ppb; 190 had TCE exposure of 501−5,000 ppb; 35 had TCE exposure >5,000 ppb.	Interviews (occupational, environmental, demographic, and health information); A large number of health outcomes analyzed, including speech impairment and hearing impairment.	Logistic Regression, Odds Ratios; lowest quartile used as reference population.	When the registrants were grouped by duration of exposure to TCE, a statistically significant association (adjusted for age and sex) between duration of exposure and reported hearing impairment was found. The prevalence odds ratios were 2.32 (95% Cl: 1.18, 4.56) (>2 to <5 yrs); 1.17 (95% Cl: 0.55, 2.49) (>5 to <10 yrs); and 2.46 (95% Cl:1.30, 5.02) (>10 yrs); Higher rates of speech impairment (not statistically significant) associated with maximum and cumulative TCE exposure, and duration of exposure.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Buxton and Hayward, 1967	This was a case study on 4 workers exposed to very high concentrations of TCE, which resulted from an industrial accident. No controls were evaluated.	Case 1 was a 44-yr old man exposed for 10 min; Case 2 was a 39-yr old man exposed for 30 min; Case 3 was a 43-yr old man exposed for 2.5 h; Case 4 was a 39-yr old man exposed for 4 h. TCE concentrations were not reported.	Clinical evaluations were conducted by a physician when patients presented with symptoms; numbness of face, ocular pain, enlarged right blind spot, nausea, loss of taste, headache, dizziness, unsteadiness, facial diplesia, loss of gag and swallowing reflex, absence of corneal reflex, and reduction of trigeminal response.	There was no statistical assessment of results presented.	Case 1 exhibited headaches and nausea for 48 h, but had a full recovery. Case 2 exhibited nausea and numbness of face, but had a full recovery. Case 3 was seen and treated at a hospital with numbness of face, insensitivity to pin prick over the trigeminal distribution, ocular pain, enlarged right blind spot, nausea, and loss of taste. No loss of mental faculty was observed. Case 4 was seen and treated for headache, nausea, dizziness, unsteadiness, facial diplesia, loss of gag and swallowing reflex, facial analgesia, absence of corneal reflex, and reduction of trigeminal response. The patient died and was examined postmortem. There was demyelination of the 5th cranial nerve evident.
Chalupa et al., 1960	This was a case study conducted on 22 patients with acute poisoning caused by carbon monoxide and industrial solvents. Six subjects were exposed to TCE (doses not known). Average age 38.	No exposure data were reported.	Medical and psychological exams were given to all subjects. These included EEGs, measuring middle voltage theta activity of 5−6 sec duration. Subjects were tested for memory disturbances.	No statistics were performed.	80% of those with pathological EEG displayed memory loss; 30% of those with normal EEGs displayed memory loss. Pathology and memory loss were most pronounced in subjects exposed to carbon monoxide.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
El Ghawabi et al., 1973	30 money printing shop workers occupationally exposed to TCE; Controls: 20 age and SES matched nonexposed males and 10 control workers not exposed to TCE but exposed to inks used in printing.	Air samples on 30 workers. Mean TCE air concentrations ranged from 41 to 163 ppm throughout the Intalgio process Colorimetric determination of both TCA and total trichloro-compounds in urine with Fujiware reaction.	Inquiries about occupational, past and present medical histories, and family histories in addition to age and smoking habits. EKGs were performed on 25 of the workers. Lab investigations included complete blood and urine analysis, and routine liver function tests.	Descriptive statistics and central tendency evaluation for metabolites; no stats reported for neurological symptoms.	Most frequent symptoms: prenarcotic headache (86% vs. 30% for controls), dizziness (67% vs. 6.7% for controls), and sleepiness (53% vs. 6% for controls) main presenting symptoms in addition to suppression of libido. Trigeminal nerve involvement was not detected. The concentration of total trichloro-compounds increased toward mid-week and was stationary during the last 2 working days. Metabolites of total trichloroacetic acid and trichloroethanol are only proportional to TCE concentrations up to 100 ppm.
Feldman et al., 1988	21 Massachusetts residents with alleged chronic exposure to TCE in drinking water; 27 laboratory controls.	TCE in residential well water was 30−80 times greater than U.S. EPA MCL; maximum reported concentration was 267 ppb; other solvents also present.	BR used as an objective indicator of neurotoxic effects of TCE; clinical neurological exam, EMGs to evaluate blink reflex, nerve conduction studies, and extensive neuropsychological testing.	Student's t-test used for testing the difference between the group means for the Blink reflex component latencies.	Highly significant differences in the conduction latency means of the BR components for the TCE exposed population vs. control population, when comparing means for the right and left side R1 to the controls ($p < 0.001$). The mean R1 BR component latency for the exposed group was 11.35 ms, SD = 0.74 ms, 95% CI: 11.03−11.66. The mean for the controls was 10.21 ms, SD = 0.78 ms, 95% CI: 9.92−10.51; $p < 0.001$. Suggests a subclinical alteration of the trigeminal nerve function due to chronic, environmental exposure to TCE.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Feldman et al., 1992	18 workers occupationally exposed to TCE; 30 laboratory controls.	Reviewed exposure histories of each worker (job type, length of work) and audited medical records to categorize into three exposure categories: “extensive,” “occasional,” and “chemical other than TCE”.	Blink reflexes using TECA 4 EMG.	Non-Gaussian distribution and high coefficient of variance data were log-transformed and then compared to the log-transformed control mean values. MRV was calculated by subtracting the subjects value (*x*) from the control group mean (M), and the difference is divided by the control group standard deviation.	The “extensive” group revealed latencies greater than 3 SD above the nonexposed group mean on R1 component of blink reflex; none of the “occasional” group exhibited such latencies, however, two of them demonstrated evidence of demyelinating neuropathy on conduction velocity studies; the sensitivity, or the ability of a positive blink reflex test to correctly identify those who had TCE exposure, was 50%. However, the specificity was 90%, which means that of those workers with no exposure to TCE, 90% demonstrated a normal K1 latency. Subclinical alteration of the Vth cranial nerve due to chronic occupational exposure to TCE is suggested.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Gash et al., 2007	30 Parkinson's Disease patients and 27 non-Parkinson coworkers exposed to TCE; No unexposed controls.	Mapping of work areas.	General physical exam, neurological exam and UPDRS, timed motor tests, and occupational history survey; mitochondrial neurotoxicity; Questionnaire mailed to 134 former non-Parkinson's workers, (14 symptomatic of parkinsonism: age range = 31−66, duration of employment range: 11−35 yrs) (13 asymptomatic: age range = 46−63, duration of employment range: 8−33 yrs);.	Workers' raw scores given; ANOVA comparing symptomatic vs. nonsymptomatic workers.	Symptomatic non-Parkinson's group was significantly slower in fine motor hand movements than age-matched nonsymptomatic group ($p < 0.001$); All symptomatic workers had positive responses to 1 or more questions on UPDRS Part I and Part II, and/or had signs of parkinsonism on Part III; One symptomatic worker had been tested 1 yr. prior and his UPDRS score had progressed from 9 to 23.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Grandjean et al., 1955	80 workers employed in 10 different factories of the Swiss mechanical engineering industry exposed to TCE, seven of whom stopped working with TCE from 3 wks to 6 yrs prior; no unexposed control group.	Vapors were collected in ethylic alcohol 95%. Volume of air was checked using a flowmeter, and quantitatively measured according to the method of Truhaut (1951), which is based on a colored reaction between TCE and the pyridine in an alkaline medium (with modifications). Urine analysis of TCA levels; TCE air concentrations varied from 6−1,120 ppm depending on time of day and proximity to tanks, but mainly averaged between 20−40 ppm. Urinalysis varied from 30 mg/L to 300 mg/L; Could not establish a relationship between TCE eliminated through urine and TCE air levels. Four exposure groups estimated based on air sampling data.	Medical exam, including histories; Blood and biochem. tests, and psychiatric exam. Psychological exam; Meggendorf, Bourdon, Rorschach, Jung, Knoepfel's "thirteen mistakes" test, and Bleuler's test.	Coefficient of determination, Regression coefficient.	Men working all day with TCE showed on average larger amounts of TCA than those who worked part time with TCE. Relatively high frequency of subjective complaints, of alterations of the vegetative nervous system, and of neurological and psychiatric symptoms. 34% had slight or moderate psycho-organic syndrome; 28% had neurological changes; There is a relationship between the frequency of those alterations and the degree of exposure to TCE. There were significant differences ($p = 0.05$) in the incidence of neurological disorder between Groups I and III, while between Groups II and III there were significant differences ($p = 0.05$) in vegetative and neurological disorders. Based on TCA eliminated in the urine, results show that subjective, vegetative, and neurological disorders were more frequent in Groups II and III than in Group I. Statistical analysis revealed the following significant differences ($p < 0.01$): subjective disorders between I and II ; vegetative disorders between I and II and between I and III; neurological disorders between I and (II and III). Vegetative, neurological, and psychological symptoms increased with the length of exposure to TCE. The following definite differences were shown by statistical analysis ($p < 0.03$) : vegetative disorders between I and IV ; neurological disorders between I and II and between I and IV; psychological disorders between I and III and between I and IV.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Gun, el al., 1978	8 exposed: 4 female workers from one plant exposed to TCE and 4 female workers from another plant exposed to TCE + nonhalogenated hydrocarbon solvent used in degreasing; control group (*n* = 8) consisted of 4 female workers from each plant who did not work near TCE.	Air sampled continuously over separate 10 min durations drawn into a Davis Halide Meter. Readings taken every 30 sec.; ranged from 3−419 ppm.	Eight-Choice reaction times carried out in four sessions; 40 reaction time trials completed.	Variations in RT by level of exposure; ambient air exposure TCE concentrations and mean air TCE values.	TCE only group had consistently high mean ambient air TCE levels (which exceeded the 1978 TLV of 100 ppm) and showed a mean increase in reaction time, with a probable cumulative effect. In TCE + solvent group, ambient TCE was lower (did not exceed 100 ppm) and mean reaction time shortened in Session 2, then rose subsequently to be greater than at the start. Both control groups showed a shortening in mean choice reaction time in Session 2 which was sustained in Sessions 3 and 4 consistent with a practice effect; No stats provided.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Hirsch et al., 1996	106 residents of Roscoe, a community in Illinois on the Rock River, in direct proximity to an industrial plant that released an unknown amount of TCE into the River. All involved in litigation. Case series report; No unexposed controls.	Random testing of the wells between 1983−84 revealed groundwater in wells to have levels of TCE between 0 to 2,441 ppb; distance of residence from well used to estimate exposure level.	Medical, neurologic, and psychiatric exams and histories. For those who complained of headaches, a detailed headache history was taken, and an extensive exam of nerve-threshold measurements of toes, fingers, face, olfactory threshold tests for phenylethyl methylethyl carbinol, brain map, Fast Fourier Transform (FFT), P300 Cognitive auditory evoked response, EEG, Visual Evoked Response (VER), Somato sensory Evoked Potential (SSER), Brainstem Auditory Evoked Response (BAER), MMPI-II, MCMI-II, and Beck Depression Inventory were also given.	Student t-test, Chi square analysis, nonparametric t-test and ANOVA, correlating all history, physical exam findings, test data, TCE levels in wells, and distance from plant.	66 subjects (62%) complained of headaches, Diagnosis of TCE-induced cephalagia was considered credible for 57 patients (54%). Retrospective TCE level of well water or well's distance from the industrial site analysis did not correlate with the occurrence of possibly-TCE induced headaches. Studies that were not statistically significant with regard to possible TCE-cephalalgia included P300, FFT, VER, BAER, MMPI, MCMI, Beck Depression Inventory, SSER, and nerve threshold measurements. Headache might be associated with exposure to TCE at lower levels than previously reported. Headaches mainly occurred without sex predominance, gradual onset, bifrontal, throbbing, without associated features; No quantitative data presented to support statement of headache in relation to TCE exposure levels, except for incidences of headache reporting and measured TCE levels in wells.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn and Thornton, 1996	Group A: Randomly selected registered voters from Arizona and Louisiana with no exposure to TCE: *n* = 264 unexposed volunteers aged 18−83: Group B volunteers from California *n* = 29 17 males and 12 females to validate the equations; Group C exposed to TCE and other chemicals residentially for 5 yrs or more *n* = 237.	No exposure or groundwater analyses reported.	Reaction speed using a timed computer visual-stimulus generator; Compared groups to plotted measured SRT and CRT Questionnaire to eliminate those exposed to possibly confounding chemicals.	Box-Cox transformation for dependent and independent variables. Evaluated graphical methods to study residual plots. Cooks distance statistic measured influence of outliers examined. Lack-of-fit test performed on Final model and F statistic to compare estimated error to lack-of-fit component of the model's residual sum of squared error. Final models were validated using Group B data and paired t-test to compare observed values for SRT and CRT. F statistic to test hypothesis that parameter estimates obtained with Group B were equal to those of the model.	Group A: SRT = 282 ms CRT = 532 ms Group B: SRT = 269 ms CRT = 531 ms Group C: SRT = 334 ms CRT = 619 ms Lg(SRT) = 5.620, SD = 0.198 Regression equation for Lg(CRT) = 6.094389 + 0.0037964 × age. TCE exposure produced a step increase in SRT and CRT, but no divergent lines. Coefficients from Group A were valid for Group B. Predicted value for SRT and for CRT, plus 1.5 SDs. selected 8% of the model group as abnormal.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn and Warshaw, 1993	Well-water exposed subjects to 6 to 500 ppb of TCE for 1 to 25 yrs; 544 recruited test subjects; Group 1 = 196 exposed family members of subjects with cancer or birth defects; Group 2 = 178 from exposed families without cancer or birth defects; Group 3 = 170 exposed parents whose children had birth defects and rheumatic disorders; Controls: 68 referents and 113 histology technicians (HTs) without environmental exposure to TCE.	Well-water was measured from 1957 to 1981 by several governmental agencies, and average annual TCE exposures were calculated and then multiplied by each individual's years of residence for 170 subjects.	Neurobehavioral testing - augmented NBT; Eye Closure and Blink using EMG; neuropsychological (NPS) test - Portions of Wechsler's Memory Scale, and WAIS and embedded figures test, grooved pegboard, Trail Making A and B, POMS, and Culture Fair Test; neurophysiological (NPH) testing - Simple visual reaction time, body balance apparatus, cerebellar function, proprioception, visual, associative links and motor effector function.	Two sided student t-test with a $p < 0.05$. Linear regression coefficients to test how demographic variables or other factors may contribute.	Exposed subjects had lower intelligence scores and more mood disorders. NPH: Significant impairments in sway speed with eyes open and closed, blink reflex latency (R-1), eye closure speed, and two choice visual reaction time. NPS: Significant impairments in Culture Fair (intelligence) scores, recall of stories, visual recall, digit span, block design, recognition of fingertip numbers, grooved pegboard, and Trail Making A and B. POMS: all subtests, but the fatigue, were elevated Mean speeds of sway were greater with eyes open at $p < 0.0001$) and with eyes closed $p < 0.05$) in the exposed group compared to the combined referents. The exposed group mean simple reaction time was 67 msec longer than the referent group $p < 0.0001$). Choice reaction time (CRT) of the exposed subjects was 93 msec longer in the third trial ($p < 0.0001$) than referents. It was also longer in all trials, and remained significantly different after age adjustment. Eye closure latency was slower for both eyes in the exposed and significantly different ($p < 0.0014$) on the right compared to the HT referent group.
Kilburn, 2002b	236 residents chronically exposed to TCE and associated solvents, including DCE, PCE, and vinyl chloride, in the environment from a	Exposure estimate based on groundwater plume based on contour mapping; concentrations between 0.2−10,000 ppb of TCE over a 64 km^2 area; additional	Simple reaction time, choice reaction time, Balance sway speed (with eyes open and eyes closed), color errors, blink reflex latency, Supra orbital	Descriptive statistics; ANCOVA; step-wise adjustment of demographics.	The principal comparison, that was between the 236 exposed persons and the 161 unexposed regional controls, revealed 13 significant differences ($p < 0.05$). SRTs and CRTs were delayed. Balance was abnormal with excessive sway speed (eyes closed), but this was not true when both eyes were open. Color discrimination

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn, 2002b (continued)	nearby microchip plant, some involved in litigation, prior to 1983 and those who lived in the area between 1983 and 1993 during which time dumping of chlorinated solvents had supposedly ceased and clean-up activities had been enacted; Controls: 67 referents from northeast Phoenix, who had never resided near the 2 plants (mean distance = 2,000 m, range = 1,400−3,600 m from plants) and 161 regional referents from Wickenburg, AZ up-wind of Phoenix, recruited via random calls made to numbers on voter registration rolls, matched to exposed subjects by age and years of education, records showed no current or past water contamination in the areas.	associated solvents, including DCE, PCE, and vinyl chloride, No air sampling.	tap (left and right), Culture Fair A, Vocabulary, Pegboard, Trail Making A and B, Immediate verbal recall, POMS; Pulmonary Function; The same examiners who were blinded to the subjects' exposure status examined the Phoenix group, but the Wickenburg referents' status was known to the examiners. Exact order or timing of testing not stated.		errors were increased. Both right and left blink reflex latencies (R-1) were prolonged. Scores on Culture Fair 2A, vocabulary, grooved pegboard (dominant hand), trail making A and B, and verbal recall (i.e., memory) were decreased in the exposed subjects. Litigation is suggested but not stated and study paid by lawyers. Litigation status may introduce a bias, particularly if no validity tests were used.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kilburn, 2002b	236 residents exposed environmentally from a nearby microchip plant (exact number of litigants not stated); 156 individuals exposed for >10 y compared to 80 individuals <10 y of exposure; Controls: 58 nonclaimants in 3 areas within exposure zone (Zones A, B, and C).	No discussion of exposure assessment methods and results. Solvents included TCE, DCE, PCE, and vinyl chloride; concluded exposure is primarily due to groundwater plume rather than air releases.	Simple reaction time, choice reaction time, Balance sway speed (with eyes open and eyes closed), color errors, blink reflex latency, Supra orbital tap (left and right), Culture Fair A, Vocabulary, Pegboard, Trail Making A and B, Immediate verbal recall, POMS.	Descriptive statistics, Regression analysis; Similar study to the one reported above with the exception of looking at the effects of duration of residence, proximity to the microchip plant, and being involved in litigation.	Insignificant effects of longer duration of residence. No effect of proximity and litigation. Effects of longer duration of residence modest and insignificant. No effect of proximity. No litigation effect. Zone A- 100 clients were not different from the 9 nonclients. Zone B, nonclients were more abnormal in color different than clients and right-sided blink was less abnormal in nonclients. Zone C, 9 of the 13 measurements were not significantly different. 26 of the original 236 subjects re-tested in 1999: maintained impaired levels of functioning and mood; No tests of effort and malingering used, limiting interpretations. Again, no tests of effort and malingering were used, thus, limiting interpretation. Litigation is suggested but not stated and study paid by lawyers. Litigation status may introduce a bias, particularly if no validity tests were used.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Landrigan et al., 1987	13 Pennsylvania residents exposed through drinking and bathing water contaminated by approximately 1,900 gallon TCE spill; Feb 1980: 9 workers exposed to TCE while degreasing metal in pipe manufacturing plant and 9 unexposed controls (mean ages were 42.7 exposed and 46.4-y old unexposed; mean durations of employment = 4.4, exposed, and 9.4 y, unexposed.; May 1980: 10 exposed workers and same 9 unexposed worker controls from Feb monitoring.	Community Evaluation: Nov 1979- Questionnaires on TCE and other chemical exposures, and occurrence of signs and symptoms of exposure to TCE, morning urine samples, urine samples analyzed coloreimetrically for total trichloro-compounds. Occupational Evaluations (In workers): breathing-zone air samples(mean 205 mg/m^3; 37 ppm); medical evaluations, pre and post shift spot urine samples in Feb and again in May, mid and post shift venous blood samples during the May survey,	Community evaluation, occupational evaluations; urine evaluations for TCE metabolites; Questionnaires to evaluate neurologic effects and symptoms; ISO concentrations, Map of TCE in groundwater.	Descriptive statistics	Community Evaluation: No urinary TCA detected in community population except for 1 resident also working at plant and 1 resident with no exposure; Occupational Evaluation: Range 117−357 mg/m^3 – (21−64 ppm). Feb: airborne exposures exceeded NIOSH limit by up to 222 mg/m^3 (40 ppm)(NIOSH TWA <135 mg/m$^{3)}$. (24 ppm). Short term exposure exceeded NIOSH values of 535 mg/m^3 (96 ppm) by up to 1,465 mg/m^3 (264 ppm). Personal breathing zone of other workers within recommended limits (0.5−125 mg/m^3) (0.1−23 ppm). 7 exposed workers reported acute symptoms, including fatigue, light-headedness, sleepiness, nausea, headache, consistent with TCE exposure; No control workers reported such symptoms; Prevalence of 1 or more symptoms 78% in exposed worker group, 0% in control worker group; Symptoms decreased after recommendations were in place for 3 mos (may testing) for reduced exposures.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Liu et al., 1988	103 workers from factories in Northern China, exposed to TCE (79 men, 24 women), during vapor degreasing production or operation. The unexposed control group included 85 men and 26 women.	Exposed to TCE, mostly at less than 50 ppm; concentration of breathing zone air during entire shift measured by diffusive samplers placed on the chest of each worker; divided into three exposure groups; 1−10 ppm, 11−50 ppm and 51−100 ppm; Also, hematology, serum biochemistry, sugar, protein, and occult blood in urine were collected.	Self-reported subjective symptom questionnaire.	Prevalence of affirmative answers = total number of affirmative answers divided by (number of respondents × number of questions); X^2.	Dose-response relationship established in symptoms such as nausea, drunken feeling, light-headedness, floating sensation, heavy feeling of the head, forgetfulness, tremors and/or cramps in extremities, body weight loss, changes in perspiration pattern, joint pain, and dry mouth (all $\geq$3 times more common in exposed workers); "*bloody strawberry jam-like feces"* was borderline significant in the exposed group and "*frequent flatus"* was statistically significant. Exposure ranged up to 100 ppm, however, most workers were exposed below 10 ppm, and some at 11−50 ppm. Contrary to expectations, production plant men had significantly higher levels of exposure (24 had levels of 1−10 ppm, 15 had levels of 11−50 ppm, 4 had levels of 51−100 ppm) than degreasing plant men (31 had levels of 1−10 ppm, 2 had levels of 11−50 ppm, 0 had levels of 51−100 ppm); $p < 0.05$ by chi-square test. No significant difference ($p > 0.10$) was found in women workers. The differences in exposure intensity between men and women was of borderline significance ($0.05 < p < 0.10$).

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
McCunney, 1988	This is a case study conducted on 3 young white male workers exposed to TCE in degreasing operations. There were no controls included. Case 1 was a 25-yr old male, Case 2 was a 28-yr old white male, Case 3 was a 45-yr old white male.	Case 1: TCE in air at the work place was measured at 25 ppm, but his TCA in urine was measured at 210 mg/L. This is likely due to dermal exposure while cleaning metal rods in TCE. Case 2: no TCE exposure data presented, TCA at 9 mg/L after 6 mos; Case 3: no TCE exposure data presented.	Clinical evaluation of loss of balance, light headedness, resting tremor, blurred vision, and dysdiadochokinesia, change in demeanor and loss of coordination, cognitive changes were noted, as well as depression; CT scan, EEG, nerve conductivity, and visual and somatosensory evoked response. Neurological exams included sensitivity to pinprick over the face; Ophthalmic evaluation.	There were no statistical analyses of results presented.	Case 1 was a 25-yr old male, who presented with a loss of balance, light headedness, resting tremor, blurred vision, and dysdiadochokinesia. The subject had been in a car accident and suffered head injuries. He later returned with a change in demeanor and loss of coordination. He showed a normal CT scan, EEG, nerve conductivity, and visual and somatosensory evoked response. Neurological exams revealed reduced sensitivity to pinprick over the face, deep tendon reflexes were reduced, mild to moderate cognitive changes were noted, as well as depression. Ophthalmic evaluation was normal. He was removed form the TCE exposure and appeared to recover. Case 2 was a 28-yr old white male who presented with numbness and shooting pains in fingers. He exhibited anorexia, tiredness. He worked in a degreasing operation for a jeweler using open containers filled with TCE in a small, unventilated room. There were no exposure data provided, but his TCA was 9 mg/L at 6 mos after exposure. He had been hospitalized with hepatitis previously. No neurological tests were administered. Case 3 was a 45-yr old white male who presented with numbness in hands and an inability to sleep. He exhibited slurred speech. He was positive for blood in stool, but had a history of duodenal ulcers.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Mhiri et al., 2004	23 phosphate industry workers exposed to TCE for 6 h/d for at least 2 yrs while cleaning walls to be painted; Controls: 23 unexposed workers from the department of neurology.	Measurement of urinary metabolites of TCE were performed 3 times/worker. Blood tests and hepatic enzymes were also collected.	Trigeminal somatosensory evoked potentials recorded using Nihon-Kohden EMG- evoked potential system; baseline clinical evaluations regarding facial burn or numbness, visual disturbances, restlessness, concentration difficulty, fatigue, mood changes, assessment of cranial nerves, quality of life; biological tests described under biomarkers.	Paired or unpaired Student's t-test as appropriate. *p*-value set at <0.05. Spearman rank-correlation procedure was used for correlation analysis.	Abnormal TSEP were observed in 6 workers with clinical evidence of Trigeminal involvement and in 9 asymptomatic workers. A significant positive correlation between duration of exposure and the N2 latency ($p < 0.01$) and P2 latency ($p < 0.02$) was observed. Only one subject had urinary TCE metabolite levels over tolerated limits. TCE air contents were over tolerated levels, ranging from 50−150 ppm.
Mitchell and Parsons-Smith, 1969	This was a case study of 1 male patient, age 33, occupational exposed to TCE during degreasing. There were no controls.	No exposure data are presented.	Trigeminal nerve, loss of taste, X-rays of the skull, EEG, hemoglobin, and Wassermann reaction.	No statistics provided.	The patient had complete analgesia in the right trigeminal nerve and complete loss of taste, patient complained of loss of sensation on right side of face, and uncomfortable right eye, as well as vertigo and depression. X-rays of the skull, EEG, hemoglobin, and Wassermann reaction were all normal.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Nagaya et al., 1990	84 male workers ages 18−61 (mean 36.2) constantly using TCE in their jobs. Duration of employment (i.e., exposure) 0.1−34.0 yrs, (mean 6.1 yrs; SD = 5.9). Controls: 83 age-matched office workers and students with no exposure.	Workers exposed to about 22-ppm TCE in air. Serum dopamine-β-hydroxylase (DBH) activity levels measured from blood. Urinary total trichloro-compounds (U-TTC) also measured.	Blood drawn during working time and DBH activities were analyzed; Spot urine collected at time of blood sampling and U-TTC determined by alkaline-pyridine method.	Student's t-test and linear correlation coefficient. Results of U-TTC presented by age groups: ≤25; 26−40; ≥41.	A slight decrease in serum DBH activity with age was noted in both groups. Significant inverse correlation of DBH activity and age was found in workers (r = −0.278, $0.01 < p < 0.02$), but not in controls (r = −0.182, $0.05 < p < 0.1$). No significant differences between mean serum DBH activity levels by age groups for workers and corresponding controls in any age group. Workers' U-TTC levels: 3.8 to 1,066.4 mg/L (M = 133.6 mg/L); U-TTC not detected in controls. Serum DBH activity levels in workers independent of U-TTC levels and duration of employment. Results suggest that chronic occupational exposure to TCE did not influence sympathetic nerve activity.
Reif et al., 2003	143 residents of the Rocky Mountain Arsenal community of Denver whose water was contaminated with TCE and related chemicals from nearby hazardous waste sites between 1981 and 1986; Referent group at lowest concentration (<5 ppb).	Hydraulic simulation model used in conjunction with a GIS estimated residential exposures to TCE; Approximately 80% of the sample exposed to TCE exceeding MCL of 5 ppb and approximately 14% exceeded 15 ppb. High exposure group >15 ppb, low exposure referent group <5 ppb, medium exposure group $5 < x < 15$ ppb.	NCTB, tests of visual contrast sensitivity, POMS.	Multivariate Model.	Statistical significance was approached as a result of high TCE exposure vs. referent group; poorer performance on the digit symbol ($p = 0.07$), contrast sensitivity C test ($p = 0.06$), and contrast sensitivity D test ($p = 0.07$), and higher mean scores for depression ($p = 0.08$). Alcohol was an effect modifier in high-exposed individuals—statistically significant on the Benton, digit symbol, digit span, and simple reaction time tests, as well as for confusion, depression, and tension.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen and Sabroe, 1986	368 metal workers working in degreasing at various factories in Denmark (industries not specified) with chlorinated solvents; 94 controls randomly selected semiskilled metal workers from same area; mean age: 37.7 (range: 17−65+). Total 443 men; 19 women.	Questionnaire: categorized in 4 groups; 3 exposure groups plus control: (1) currently working with chlorinated solvents (*n* = 171; average. duration: 7.3 yrs, 16.5 h/wk; 57% TCE and 37% 1,1,1-trichloroethane), (2) currently working with other solvents (*n* = 131; petroleum, gasoline, toluene, xylene), (3) previously (1−5 yrs) worked with chlorinated or other solvents (*n* = 66) (4) never worked with organic solvents (*n* = 94).	Questionnaire: 74 items about neuropsychological symptoms (memory, concentration, irritability, alcohol intolerance, sleep disturbance, fatigue).	Chi-square; Odds ratios; t-test; logistic regression.	Neuropsychological symptoms significantly more prevalent in the chlorinated solvents-exposed group; TCE caused the most "inconveniences and symptoms;" dose response between exposure to chlorinated solvents and chronic neuropsychological symptoms (memory [$p < 0.001$], concentration [$p < 0.02$], irritability [$p < 0.004$], alcohol intolerance [$p < 0.004$], forgetfulness [$p < 0.001$], dizziness [$p < 0.005$], and headache [$p < 0.01$]); Significant associations between previous exposure and consumption of alcohol with chronic neuropsychological symptom.s

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al., 1993a	96 Danish workers involved in metal degreasing with chlorinated solvents, mostly TCE (n = 70); (industries not specified), age range: 19−68; no external controls.	Chronic exposure to TCE (n = 70); CFC (n = 25); HC (n = 1); average duration: 7.1 yrs; range of full-time degreasing: 1 mo to 36 yrs; occupational history, blood and urinary metabolites (TCA); biological monitoring for TCE and TCE metabolites; CEI calculated based on number of h/wk worked with solvents × yr of exposure × 45 wk per yr; 3 groups: (1) low exposure: n = 19, average full-time exposure 0.5 yr; (2) medium exposure: n = 36, average full-time exposure 2.1 yrs.; (3) high exposure: n = 41, average full-time exposure 11 yrs; Mean TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L); TWA measurements of CFC 113 levels: 260−420 ppm (U.S. and Danish TLV is 500 ppm).	Medical interview, neurological exam, neuropsychological exam; **Tests:** WAIS: Vocabulary, Digit Symbol; Simple Reaction Time, acoustic-motor function, discriminatory attention, Sentence Repetition, Paced Auditory Serial Addition Test, Text Repetition, Rey's Auditory Verbal Learning, visual gestalt, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, Mira; Blind study.	Fisher's exact test, Chi-square trend test, t-test, ANOVA, logistic regression, odds ratios, Chi-square goodness-of-fit test; Confounders examined: age, primary intellectual level, arteriosclerosis, neurological/psychiatric disease, alcohol abuse, and present solvent exposure.	After adjusting for confounders, the high exposure group has significantly increased risk for psychoorganic syndrome following exposure (OR: 11.2); OR for medium exposed group = 5.6; Significant increase in risk with age and with decrease in WAIS Vocabulary scores; Prevalence of psychoorganic syndrome: 10.5% in low exposure group, 38.9 in medium exposure group, 63.4% in high exposure group; no significant interaction between age and solvent exposure.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al., 1993b	96 Danish workers involved in metal degreasing with chlorinated solvents (industries not specified), age range: 19−68; No external controls.	Chronic exposure to TCE (n = 70); CFC (n = 25); HC (n = 1); average duration: 7.1 yrs); range of full-time degreasing: 1 mo to 36 yrs; occupational history, blood and urinary metabolites (TCA); biological monitoring for TCE and TCE metabolites; CEI calculated based on number of h/wk worked with solvents × yr of exposure × 45 wks per yr; 3 groups: (1) low exposure: n = 19, average full-time expo 0.5 yr; (2) medium exposure: n = 36, average full-time exposure 2.1 yrs; (3) high exposure: n = 41, average full-time exposure 11 yrs; Mean TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L); TWA measurements of CFC 113 levels: 260−420 ppm (U.S. and Danish TLV is 500 ppm).	WAIS (original version): Vocabulary, Digit Symbol, Digit Span; Simple Reaction Time, Acoustic-motor function (Luria), Discriminatory attention (Luria), Sentence Repetition, PASAT, Text Repetition, Rey's Auditory Verbal Learning, Visual Gestalts, Stone Pictures (developed for this study, nonvalidated), revised Santa Ana, Luria motor function, Mira; Blind study.	Linear regression analysis; Confounding variables analyzed: age, primary intellectual function, word blindness, education, arteriosclerosis, neurological/psychiatric disease, alcohol use, present solvent exposure.	Dose response with 9 of 15 tests; Controlling for confounds, significant relationship of exposure was found with Acoustic-motor function ($p < 0.001$), PASAT ($p < 0.001$), Rey AVLT ($p < 0.001$), vocabulary ($p < 0.001$), and visual gestalts ($p < 0.001$); significant age effects.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Rasmussen et al., 1993c	96 Danish workers involved in metal degreasing with chlorinated solvents (industries not specified), age range: 19−68; No external controls.	Chronic exposure to TCE (*n* = 70); CFC (*n* = 25); HC (*n* = 1); average duration: 7.1 yrs); range of full-time degreasing: 1 mo to 36 yrs; occupational history, blood and urinary metabolites; biological monitoring for TCE and TCE metabolites; CEI calculated based on number of h/wk worked with solvents × yr of exposure × 45 wk per yr; 3 groups: (1) low exposure: *n* = 19, average full-time expo 0.5 yr; (2) medium exposure: *n* = 36, average full-time exposure 2.1 yrs; (3) high exposure: *n* = 41, average full-time exposure 11 yrs; Mean TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L); TWA measurements of CFC 113 levels: 260−420 ppm (U.S. and Danish TLV is 500 ppm).	Medical interview, clinical neurological exam, neuropsychological exam.	Multiple regression; Fisher's exact test; Mantel-Haenzel test for linear association.	Significant dose response between exposure and motor dyscoordination remained after controlling for confounders; Bivariate analysis showed increased vibration threshold with increased exposure, but with multivariate analysis, age was a significant factor for the increase.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Ruijten et al., 1991	31 male printing workers exposed to TCE. Mean age 44; Mean duration 16 yrs; Controls: 28; mean age 45 yrs.	Relied on exposure data from past monitoring activities conducted by plant personnel using gas detection tubes. Estimated 17 ppm for past 3 yrs, 35 ppm for preceding 8 yrs and 70 ppm before that. Individual cumulative exposure was calculated as time spent in different exposure periods and the estimated exposure in those periods. Mean cumulative exposure = 704 ppm × yrs (SD 583, range: 160−2,150 ppm × yrs.	General questionnaire, cardiotachogram recorded on ink writer to measure Autonomic nerve function, including forced respiratory sinus arrhythmia (FRSA), muscle heart reflex (MHR), resting arrhythmia; Trigeminal nerve function measured using masseter reflex and blink reflex; electrophysiological testing of peripheral nerve functioning using motor nerve conduction velocity of the peroneal nerve.	Combined Z score = individual Z scores of the FRSA and MHR; ANCOVA to calculate difference between exposed/nonexposed workers; Cumulative exposure effect calculated by multiple linear regression analysis. Controlled for age, alcohol consumption, and nationality by including them as covariables. Quetelet-index included for autonomic nerve parameters; Body length and skin temperature used for all peripheral nerve functions; one-sided significance level of 5% used. Non-normal distributions were log or square root transformed.	Slight reduction in Sural nerve conduction velocity was found and a prolongation of the Sural refractory period. Latency of the masseter reflex had increased. No prolongation of the blink reflex was found; no impairment of autonomic or motor nerve function were found. Long term exposure to TCE at threshold limit values (approximately 35 ppm) may slightly affect the trigeminal and sural nerves.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Smith, 1970	130 (108 males, 22 females); Controls: 63 unexposed men working at the same factory matched by age, marital status.	TCA metabolite levels in urine were measured: 60.8% had levels up to 20 mg/L, and 82.1% had levels up to 60 mg/L.	Cornell Medical Index Questionnaire (Psychiatric section), Heron's Personality Questionnaire, Fluency Test, 13-Mistake Test, Serial Sevens, Digit Span, General Knowledge Test, tests of memory.	Descriptive Statistics.	Of the 130 subjects exposed 27% had no complaints of symptoms, 74.5% experienced fatigue, 56.2% dizziness, 17.7% headache, 25.4% gastro-intestinal problems, 7.7% autonomic effects, and 24.9% had other symptoms. The number of complaints reported by subjects were statistically significant between those with 20 mg/L or less TCA (M = 1.8 complaints) and those 60 mg/L or more (M = 2.7). Each group, however, had a similar proportion of subjects who reported having only ‘slight’ symptoms. The total time of continuous exposure to TCE (ranging from less than 1 yr to more than 10 yrs) appeared to have little influence on frequency of symptoms. No results of the tests are reported; Author postulates that symptom assessment raises the possibility of “errors of subjective judgment.”
Triebig et al., 1977b	This study was conducted on 8 subjects occupationally exposed to TCE. Subjects were 7 men and 1 woman with an age range from 23−38 yrs. There was no control group.	Measured TCE in air averaged 50 ppm (260 mg/m^3). Length of occupational exposure was not reported.	Results were compared after exposure periods, and compared to results obtained after periods removed from exposure. TCA and TCE metabolites in urine and blood were measured. Psychological tests included d2, MWT-A, and short test.	Wilcoxon and Willcox nonparametric tests. Due to the small sample size a significance level of 1% was used.	Mean values observed were 330-mg trichloroethanol and 319-mg TCA/g creatinine, respectively, at the end of a work shift. The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig, 1982	This study was conducted on 24 healthy workers (20 males, 4 females) exposed to TCE occupationally at three different plants. The ages 17−56; length of exposure ranged from 1 to 258 mos (mean 83 mos). A control group of 144 controls used to establish 'normal' responses on the nerve conduction studies. The matched control group consisted of 24 healthy nonexposed individuals (20 males, 4 females), chosen to match the subjects for age and sex.	Length of exposure ranged from 1 to 258 mos (mean 83 mos). TCE concentrations measured in air at work places ranged from 5−70 ppm. TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine.	Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV_{MAX}(U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U), pSCV (U), and dSCV (M).	Data were analyzed using parametric and nonparametric tests, rank correlation, linear regression, with 5% error probability.	Results show no statistically significant difference in nerve conduction velocities between the exposed and unexposed groups. This study has measured exposure data, but exposures/responses are not reported by dose levels.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig, 1983	The exposed group consists of 66 healthy workers selected from a population of 112 workers. Workers were excluded based on polyneuropathy (*n* = 46) and alcohol consumption (*n* = 28). The control group consisted of 66 healthy workers with no exposures to solvents.	Subjects were exposed to a mixture of solvents, including TCE, specifically "ethanol, ethyl acetate, aliphatic hydrocarbons (gasoline), MEK, toluene, and trichloroethene." Subjects were divided into 3 exposure groups based on length of exposure, as follows: 20 employees with "short-term exposure" (7−24 mos); 24 employees with "medium-term exposure" (25−60 mos); 22 employees with "long-term exposure" (over 60 mos). TCA, TCE, and trichloroethanol were measured in blood, and TCE and TCA were measured in urine.	Nerve conduction velocities were measured for sensory and motor nerve fibers using the following tests: MCV_{MAX}(U): Maximum NLG of the motor fibers of the N. ulnaris between the wrist joint and the elbow; dSCV (U), pSCV (U), and dSCV (M).	Data were analyzed using parametric and nonparametric tests, rank correlation, linear regression, with 5% error probability.	There was a dose-response relationship observed between length of exposure to mixed solvents and statistically significant reduction in nerve conduction velocities observed for the medium and long-term exposure groups for the NCV.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Troster and Ruff, 1990	3 occupationally exposed workers to TCE or TCA: 2 patients acutely exposed to low levels of TCE and 1 patient exposed to TCA; Controls: 2 groups of *n* = 30 matched controls; (all age and education matched).	"Unknown amount of TCE for 8 months."	SDNTB, "1 or more of:" TAT, MMPI, Rorschach, and Interviewing questionnaire, Medical examinations (including neurological, CT scan, and/or Chemo-pathological tests and occupational history).	Not reported.	Case 1: Intelligence "deemed" to drop from premorbid function at 1 y 10 mos after exposure. Impaired functions improved for all but reading comprehension, visuospatial learning and categorization (abstraction). Case 2: Mild deficits in motor speed, verbal learning, and memory; "marked" deficits in visuospatial learning; good attention; diagnosis of mild depression and adjustment disorder, but symptoms subsided after removal from exposure. Case 3: Manual dexterity and logical thinking borderline impaired; no emotional changes, cognitive function spared, diagnosis of somatoform disorder.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
White et al., 1997	Group 1: 28 individuals in Massachusetts exposed to contaminated well water; source: tanning factory and chemical plant; age range: 9−55. Group 2: 12 individuals in Ohio exposed to contaminated well water; source: degreasing; age range: 12−68 Group 3: 20 individuals in Minnesota exposed to contaminated well water; *n* = 14 for nerve conduction studies and *n* = 6 for neuropsychological testing; source: ammunition plant; age range: 8−62. No controls.	Group 1: 2 wells tested in 1979: 267 ppb TCE, 21 ppb Tetrachloroethylene, 12 ppb chloroform, 29 ppb dichloro-ethylene, 23 ppb Trichlorotrifluoroethane; 2 yrs average TCE 256 ppb for well G, and 111 ppb for well H. Group 2: 13 wells with 1,1,1-trichloroethane (up to 2,569 ppb) and TCE (up to 760 ppb); blood analysis of individuals 2 yrs after end of exposure and soon after exposure showed normal or mild elevations of TCE, elevations of 1,1,1-trichloroethane, ethylbenzene, and xylenes. Group 3: mean TCE for one well 261 ppb; 1,1-dichloroethylene 9.0 ppb; 1,2-dichloroethylene 107 ppb.	Occupational and environmental questionnaire, neurological exam, neuropsychological exam: WAIS-R, WISC-R, WMS, WMS-R, Wisconsin Card Sorting, COWAT, Boston Naming, Boston Visuospatial Quantitative Battery, Milner Facial Recognition Test, Sticks Visuospatial Orientation Task, Word triads, Benton Visual Retention Test, Santa Ana, Albert's Famous Faces, Peabody Picture Vocabulary Test, WRAT, POMS, MMPI, Trail-making, Fingertapping, Delayed Recognition Span Test; Neurophysiological exam: eyeblink, evoked potentials, nerve conduction; Other: EKG, EEG, medical tests.	Data shown in proportion in 3 communities, clinical diagnostic categories, analysis of central tendencies, and descriptive statistics.	Group 1: Some individuals with subclinical peripheral neuropathy; 92.8% with reflex abnormalities; 75% total diagnosed with peripheral neuropathy; 88.9% with impairment in at least 1 memory test; Impairments: attention and executive function in 67.9%; motor function in 60.71%, visuospatial in 60.71%, mild to moderate encephalopathy in 85.7%. Group 2: 25% with abnormal nerve conduction; Impairments: attention and executive function in 83.33%, memory in 58.33%, language/verbal in 50%. Group 3: 35.7% with peripheral neuropathy; neuropsychological: all 6 tested had memory impairment, attention and executive function impairment, 3 had manual motor slowing. Participants younger at time of exposure with wider range of deficits; Language deficits in younger, but not in older participants.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Winneke, 1982	This is a review article presenting multiple studies that evaluated neurological effects of TCE, and other solvents. Only the TCE results are summarized herein. Experiment 1: 18 subjects (results taken from Schlipkoter et al., 1974 and summary is based on informations from Winneke, 1982) Experiment 2: 12 subjects (results taken from Winneke et al., 1974, 1976, 1978 and summary is based on information from Winneke, 1982)	Experiment 1: Subjects were exposed to 50 ppm TCE for 3.5 hours Experiment 2: Comparative study of effects from (a) 50 ppm TCE for 3.5 hours and (b) 0.76 ml/kg ethanol.	For both experiments 1 and 2: critical flicker fusion, sustained attention task, auditory evoked potentials	No statistical details were reported.	Significant decrease ($p < 0.05$) in auditory evoked potentials in individuals (experiments 1 and 2) exposed to 50 ppm TCE. No significant effects were noted in the critical flicker fusion or the sustained attention tasks.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
ATSDR, 2003	116 children from registry of 14 hazardous waste sites with TCE in groundwater; under 10 yrs of age at time of registry; Control population (n = 177); communities with no evidence of TCE in groundwater (measured below MCL); matched by age and race; there were other chlorinated solvents present in the exposed group wells.	Exposures were modeled using tap water TCE concentrations and GIS for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences, modeled data were used to estimate lifetime exposures (ppb-yrs) to TCE in residential wells; 3 exposure level groups; control = 0 ppb; low exposure group = 0 <23 ppb-yrs; and high exposure group = >23 ppb-yrs; confounding exposure was a concern.	Fisher Logemann test; OSME-R; CSP; D-COME-T; hearing screening; DPOAE; SCAN.	Screening results as binary variables using logistic regression within SAS; independent variables included exposure measures, age, gender, case history; chi-square test, Fisher's exact test, t-tests, linear models.	Exposed children had higher abnormalities for D-COME-T ($p < 0.002$), CSP ($p < 0.008$), velopharyngeal function ($p < 0.04$), high palatal arch ($p < 0.04$), abnormal outer ear cochlear function; No difference observed in exposed and nonexposed populations for speech or hearing function; No difference found in OSH function.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Epidemiological Studies: Controlled Exposure Studies; Neurological Effects of Trichloroethylene					
Gamberale et al., 1976	15 healthy men aged 20−31-yr old employed by the Department of Occupational Medicine in Stockholm, Sweden; Controls**:** Within Subjects (15 self-controls).	Exposed for TCE 70 mins via a breathing valve to 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), and during ordinary atmospheric air. Sequence was counterbalanced between the 3 groups, days, and exposure levels. Concentration was measured with a gas chromatographic technique every third min for the 1st 50 mins, then between tests thereafter.	RT addition, simple RT, choice RT and short term memory using an electronic panel. Subjects also assessed their own conditions on a 7-pt scale.	Friedman two-way analysis by ranks to evaluate difference between 3 conditions, nonsignificant when tested individually, but significant when tested on the basis of 6 variables. Nearly half of the subjects could distinguish exposure/nonexposure. ANOVA for 4 performance tests based on a 3 × 3 Latin square design with repeated measures.	In the RT-Addition test the level of performance varied significantly between the different exposure conditions (F[2.24] = 4.35; $p < 0.051$) and between successive measurement occasions (tF[2.24] = 19.25; $p < 0.001$); The level of performance declined with increased exposure to TCE, whereas repetition of the testing led to a pronounced improvement in performance as a result of the training effect; No significant interaction effects between exposure to TCE and training.
Konietzko et al., 1975	This is a controlled exposure study conducted on 20 healthy male students and scientific assistants with a mean age of 27.2 yrs.	Subjects were exposed to a constant TCE concentration of 95.3 ppm (520 mg/m^3) for up to 12 h, and Blood concentrations of TCE were also analyzed at hourly intervals.	Evaluated for changes in alpha waves (<14 Hz) in the EEG recordings; EEG recordings were performed hourly for a period of 1 min with the eyes closed. This was used as a potential measure of psychomotor disturbance.		The alpha segment increased over time of exposure (from 0800 to 0900 and 1000 h [military time]) (P = 0.05). There were no significant differences for the other time spans or for other parameters. Subjects with highest and lowest TCE blood levels <2 µg/mL and >5 µg/mL were compared to determine if they showed different responses, but no case were the differences statistically different.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Kylin et al., 1967	12 subjects exposed to 1,000 ppm TCE for 2 h in a 1.5 × 2 × 2 meters chamber; 2 subjects were given alcohol (0.7 gm of body weight); Controls**:** 7 of the 12 were tested some days prior to exposure and 5 of the 12 were tested some days after exposure.	1,000 ppm of TCE was blown into a chamber via an infusion unit and vaporizing system. Ostwald's distribution factor for TCE—the quotient of the amount of solvent in the blood by the amount of alveolar air.	Optokinetic Nystagmus; Venus blood and alveolar air specimens were taken at various times after exposure and analyzed in a gas chromatograph with a flame ionization detector.	Ostwald's distribution factor for TCE (the quotient of the amount of solvent in the blood in mg/L by the amount of the alveolar air in mg/L) = 9.7; Significant relationship between TCE in air and blood (0.88).	"A number" of subjects showed reduction in Fusion limit although more pronounced in the 2 subjects who consumed alcohol. "Others," however, showed little if any effect. No stats.
Salvini, 1971	This is a controlled exposure study conducted on 6 male university students. Each subject was examined on 2 different days, once under TCE exposure, and once as self controls, with no exposure.	TCE concentration was 110 ppm for 4-h intervals, twice per day. 0-ppm control exposure for all as self controls.	Two sets of tests were performed for each subject corresponding to exposure and control conditions. Perception test with tachistoscopic presentation, Wechsler memory scale, complex reaction time test (CRT), and manual dexterity test.	ANOVA	A decrease in function for all measured effects was observed. Statistically significant results were observed for perception tests learning ($p < 0.001$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$); and CRT learning ($p < 0.01$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$).

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Stewart et al., 1970	13 subjects in 10 experiments	Ten chamber exposures to TCE vapor (100 ppm and 200 ppm) for periods of 1 h to a 5-day work week. Experiments 1−7 were for a duration of 7 h with a mean TCE concentration of 198−200 ppm. Experiments 8 and 9 exposed subjects to 202 ppm TCE for a duration of 3.5 and 1 h, respectively. Experiment 10 exposed subjects to 100 ppm TCE for 4 h. Experiments 2−6 were carried out with the same subjects over 5 consecutive days; Gas chromatography of expired air; No self controls.	Physical examination 1 h prior to exposure. Blood analysis for complete blood cell count (CBC), sedimentation rate, total serum lipid, total serum protein, serum electrophoresis, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase. 24-h urine collection for urobilinogen, TCA and TCE. Also a preexposure expirogram, tidal volume measurement, and an alveolar breath sample for TCE; Short neurological exam including modified Romberg test, heel-to-toe test, finger-to-nose test.	Descriptive statistics.	Ability to perceive TCE odor diminished as duration of expo increased; 40% had dry throat after 30 min. exposure; 20% reported eye irritation; Urine specimens showed progressive increase in amounts of TCE metabolites over the 5 consecutive exposures. Concentrations of TCA and TCE decreased exponentially after last exposure, but still present in abnormal amounts in urine specimens 12 d after exposure. Loss of smelling TCE: >1 h = 33%; >2 h = 80%; >6.5 h = 100%; Symptoms of lightheadedness, headache, eye, nose and throat irritation. Prominent fatigue and sleepiness by all after 200 ppm. These symptoms may be of clinical significance. All had normal neurological tests during exposure, but 50% reported greater mental effort was required to perform a normal modified Romberg test on more than one occasion.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig, 1976	This was a controlled exposure study conducted on 7 healthy male and female students (4 females, 3 males). The control group was 7 healthy students (4 females, 3 males).	Subjects exposed for 6 h/d for 5 d to 100 ppm (550 mg/m^3 TCE). Controls were exposed in chamber to zero TCE. Biochemical tests included TCE, TCE, and trichloroethanol in blood. In this study the TCE concentrations in blood reported ranged from 4 to 14 μg/mL. A range of 20 to 60 μg/mL was obtained for TCA in the blood.	Psychological tests were: the d2 test was an attention load test; the short test is used to record patient performance with respect to memory and attention; daily Fluctuation Questionnaire measured the difference between mental states at the start of exposure and after the end of exposure is recorded; The MWT-A is a repeatable short intelligence test; the Freiburg Personality Inventory is a test for 12 independent personality traits; CFT-3 is a nonverbal intelligence test; Erlanger Depression Scale.	Regression analyses were conducted.	There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. The biochemical data did demonstrate that exposed subjects' exposures.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Triebig et al., 1977a	This was a controlled exposure study conducted on 7 healthy male and female students (4 females, 3 males) The control group was 7 healthy students (4 females, 3 males).	Subjects exposed for 6 h/d for 5 days to 100 ppm (550 mg/m^3 TCE). Controls were exposed in chamber to zero TCE. Biochemical tests included TCE, TCA and trichloroethanol in blood. In this study the TCE concentrations in blood reported ranged from 4 to 14 µg/mL. A range of 20 to 60 µg/mL was obtained for TCA in the blood.	The testing consisted of: the Syndrome Short Test; the "Attention Load Test" or "d2 Test;" Number recall test, letter recall test, The "Letter Reading Test," "Word Reading Test," Erlanger Depression Scale. Scale for Autonomic Dysfunction, Anxiety Scale, Pain Short Scale, and Information on Daily Fluctuations.	Statistics were conducted using Whitney Mann.	Results indicated the anxiety values of the placebo random sample group dropped significantly more during the course of testing ($p < 0.05$) than those of the active random sample group. No significantly different changes were obtained with any of the other variables.
Vernon and Ferguson, 1969	8 male volunteers age range 21−30; self controls: 0 dose.	TCE administered as Trilene air-vapor mixtures through spirometers administered at random concentrations of 0, 100, 300, or 1,000 ppm of TCE for 2 h at a time, during which testing took place. Concentrations were measured with a halide meter. Medical history, exam including CBC, urinalysis, BUN, and SGOT.	Flicker Fusion with Krasno-Ivy Flicker Photometer, Howard-Dolman depth perception apparatus, Muller-Lyer two-dimensional illusion, groove-type steadiness test, Purdue Pegboard, Written "code substitution," blood studies.	ANOVAs, Dunnett's test.	TCE did not produce any appreciable effects at lower concentrations. Compared to controls, participants exposed to 1,000 ppm of TCE had adverse effects on the Howard-Dolman, steadiness, and part of the pegboard, but no effects on Flicker Fusion, from perception or code substitution. No appreciable changes in CBC, urinalysis, SGOT, or BUN.

Table D-1. Epidemiological studies: neurological effects of trichloroethylene (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Windemuller and Ettema, 1978	Pilot study: 24 healthy male volunteers; age range = 19−26 yr, 4 groups with 6 volunteers in each: (1) control, (2) exposed to TCE, (3) exposed to alcohol, (4) exposed to TCE and alcohol; Final study: 15 other volunteers, each exposed to all 4 conditions.	Chamber study; Group 1 no exposure; Group 2 TCE exposure: 2.5 h with 200 ppm; Group 3 alcohol exposure: 0.35 g/kg body weight; Group 4 TCE and alcohol: same as above levels; Blood alcohol levels taken with breathalyzer; exhaled air sampled for levels of TCE and trichloroethanol; TCE exposure: average measured TCE in exhaled air = 29 μg/L (SD = 3); TCE and alcohol expo: average measured TCE in exhaled air = 63 μg/L (SD = 12).	Binary Choice Task (Visual); Pursuit Rotor; Recording of heart rate, sinus arrhythmia, breathing rate; Questionnaire (15 items on subjective feelings).	K-sample trend test; two-tailed Wilcoxon test.	Pilot study: no systematic effect of exposure on test perform. Alcohol group had higher heart rate than TCE group, and TCE and alcohol group; minimal effect of mental load on heart rate; sinus arrhythmia suppressed as mental load increased with higher suppression in exposed groups (all 3) compared to controls (differences possibly due to existing group differences); Final Study: pursuit-rotor task "somewhat impaired by exposure condition;" authors acknowledge possibility of sequence effects; no significant difference between conditions on questionnaire responses; performing mental tasks resulted in higher heart rate in the TCE + alcohol condition than in Alcohol alone condition; Mental load suppressed sinus arrhythmia, especially in TCE + alcohol condition; Conclusion: TCE and alcohol together impair mental capacity more than each one alone.

BUN = blood urea nitrogen, EEG = electroencephalograph, GI = gastrointestinal, NIOSH = National Instutute of Occupational Safety and Health, OR = odds ratio, PCE = perchloroethylene.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Albers et al., 1999	30 railroad workers with toxic encephalopathy; involved in litigation; long-term exposure to solvents (*n* = 20 yrs.; range = 10−29 yrs.); Historical controls matched by gender, age, and body mass.	Most common solvents included trichloroethylene, trichloroethane, perchloroethylene; respirator not typically used.	Neurologic exams (cranial nerves, motor function, alternate motion range, subjective sensory function, Romberg test, reflexes), occupational history, medical history, sensory and motor nerve conduction studies (NCS).	Log transformations of amplitude data; Mann-Whitney U Test for NCS; t-test; simple linear regression and stepwise regression for dose response.	3 workers met clinical polyneuropathy criteria; NCS values not influenced by exposure duration or job title; no significant difference in NCS between presence or absence of polyneuropathy symptoms, disability status, severity or type of encephalopathy, or prior polyneuropathy diagnosis.
Antti-Poika, 1982	87 patients (painters, paint and furniture factory workers, carpet and laundry workers) diagnosed 3−9 yrs prior with chronic solvent exposure (mean age 38.6 yrs) Control: 29 patients with occupational asthma.	Mean duration of exposure 10.4 yrs; solvents: trichloroethylene, perchloroethylene, solvent mixture; based on patients' and/or employers' reports; 9 worksites visited for environmental measures; biological measures at 1 worksite; exposure classified as low, moderate, or high.	Interview, Neurologic exam, EEG, electroneuromyographs, psychological examination (intellectual, short-term memory, sensory and motor functions).	Correlation coefficients for prognosis and factors influencing diagnosis.	Reported symptoms: fatigue, headaches, memory disturbances, pain, numbness, paresthesias; 1st exam: 87 patients with objective and subjective neurological signs, 61 with psychological disturbance, 58 abnormal EEG, 25 clinical abnormalities, 57 PNS symptoms; 69 patients had neurophysiological or psychological disturbances identified by neurologist in only 4 patients; 2nd exam: 42 with clinical neurological signs, ; 21 patients deteriorated, 23 improved, 43 same; poor correlation between prognosis of examinations; no significant correlation between prognosis and age, sex, exposure duration and level, alcohol use, or other diseases.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Aratani et al., 1993	437 exposed workers from various industries (not specified); 394 males, 43 females and 1,030 male clerical workers as controls; age range: 16−72.	Exposed to Thinner, G/5100, TCE, xylene, toluene, methylchloride, gasoline.	Vibrometer (VPT); Urinary Metabolites.	Spearman correlation.	Positive correlations between age and VPT 7; between job experience and VPT; Urinary metabolites not significantly correlated with VPT; no dose-effect for subjective symptoms and neurological signs.
Binaschi and Cantu, 1982	35 patients with occupational exposure to organic solvents; Industry not specified; no controls.	Occupational history provided by patients; Descriptions of jobs and conditions provided by employer; Workplace observations; Some available measurements of solvents in air; 9 patients exposed to trichloroethylene; 11 exposed to toluene and xylene; 15 exposed to mixtures of solvents; all exposures described to be under TLV-TWA, but short exposure might have exceeded ACGIF limit for short time.	Examination of provoked and spontaneous vestibular symptoms; Pure tone threshold measurement; EEG; psychiatric interviews and psychiatric history; Prevalence of 37 psychiatric symptoms.	Not stated.	All patients had subjective symptoms (fatigue, psychic disturbances, dizziness, vegetative symptoms, vertigo); Vestibular system affected in most cases, with lesions in nucleo-reticular substance and brain stem; EEG change with diffuse and focal slowing; 71% of patients had mild neurasthenic symptoms (fatigue, emotional instability, memory and concentration difficulties).

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Bowler et al., 1991	67 former microelectronics workers exposed to multiple organic solvents; Controls (*n* = 157) were recruited from the same region; 67 pairs were matched on the basis of age, sex, ethnicity, educational level, sex, and number of children.	Self-report and work history from microelectronics workers. Exposures and risks were estimated. Solvents include TCE, TCA, benzene, toluene, methylene chloride, n-hexane.	California Neuropsychological Screening Battery.	t-test for matched pairs; Wilcoxon Signed Rank test.	Exposed workers performed significantly worse on tests of attention, verbal ability, memory, visuospatial, visuomotor speed, cognitive flexibility, psychomotor speed, and reaction time; no significant differences in mental status, visual recall, learning, and tactile function.
Colvin et al., 1993	Final sample: 67 workers (43 exposed; 24 unexposed) in a paint manufacturing plant employed there for at least 5 yrs.; all black males; exclusion criteria: encephalopathy, head injury with 24 + h unconsciousness, psychotropic medication, alcohol/drug dependence history, epilepsy, mental illness.	Chronic exposure was assessed through self-reported detailed work history for each worker; past and current industrial hygiene measurements of solvent levels in air; "total cumulative expo" in the factory and "average lifetime exposures" were calculated; visitations to establish areas with "homogeneous exposure;" All exposures below the ACGIH limit. Solvents include MEK, benzene, TCE, MIBK, toluene, butyl acetate, xylene, cellosolve acetate, isophorone, and white spirits.	Work and personal history interview; brief neurological evaluation, WHO Neurobehavioral Core Test Battery (all tests except POMS); Computer-administered tests: Reaction time, Fingertapping, Continuous Performance Test, Switching attention, Pattern Recognition Test, Pattern Memory; UNISA Neuropsychological Assessment Procedure: Four word memory test, Paragraph memory, Geometric Shape drawing; symptom and health questionnaires.	Division into exposed and unexposed; Student's t-test; Multiple linear regression.	Exposed group performed worse than unexposed on 27 out of 33 test results; only significant difference was on latency times of two switching attention tests; no difference in subjects' symptom reporting between groups when questions analyzed separately or analyzed as a group; Average lifetime exposure was a significant predictor for Continuous performance latency time, Switching attention latency time, Mean reaction time, Pattern Memory; fine visuomotor tracking speed significantly associated with cumulative exposure; effects of exposure concluded to be "relatively mild" and subclinical.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Daniell et al., 1999	89 retired male workers (62−74-yr old) with prior long-term exposure to solvents including 67 retired painters and 22 aerospace manufacturing workers; Controls: 126 retired carpenters with minimal solvent exposure.	Chronic occupational exposure; Structured clinical interview about past and present exposure to solvents; Cumulative Exposure Index was constructed. Solvents not specified.	Psychiatric interview; questionnaires; physical exam; blood cell counts, chemistry panel, blood lead levels, Neuropsychological: BDI, verbal fluency test, WAIS-R: Vocabulary, Similarities, Block Design, Digit Span, Digit Symbol; Wisconsin Card Sorting; verbal aphasia screening test, Trails A and B, Fingertapping; WMS-R: logical memory and visual subtests; Rey Auditory Verbal Learning; Benton Visual Retention test; d2 test; Stroop; Grooved pegboard; simple reaction time.	Odds ratio, logarithmic transformation of non-Gaussian data, standardization of test scores, ANCOVA, Multiple Linear regression; Kruskal Wallis test for differences in blood lead concentration.	CEI was similar for painters and aerospace workers; Painters reported greater alcohol use than carpenters; painters also had lower scores on WAIS-R Vocabulary subtest; Controlling for age, education, alcohol use, and vocabulary score, painters performed worse on motor, memory, and reasoning ability tests; painters reported more symptoms of depression and neurological symptoms; painters more likely to have more abnormal test scores (odds ratio: 3.1) as did aerospace workers (odds ratio: 5.6); no dose effect with increasing exposure and neuropsychological tests.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Donoghue et al., 1995	16 patients diagnosed with organic-solvent-induced toxic encephalopathy with various occupations compared to age-stratified normal groups (*n* = 38); average age: 43 y (range = 31−58); Exclusion criteria: diabetes mellitus, ocular disease impairing vision, visual acuity with existing refractive correction of less than 4/6, abnormal direct ophthalmoscopic exam.	Average exposure duration was 19 yrs (range = 5−36 yrs); Solvents include TCE, MEK, toluene, thinners, unidentified hydrocarbons.	Visual acuity measured with a 4-m optotype chart; Contrast sensitivity measured with Vistech VCTS 6500 chart; monocular thresholds, pupil diameter.	Chi-square test.	6 participants (37.5%) with abnormal contrast sensitivity; 2 of the 6 (33%) had monocular abnormalities; abnormalities occurred at all tested spatial frequencies; significant difference between groups at 3 cpd, 6 cpd, 12 cpd frequencies.
Elofsson et al., 1980	Epidemiologic study of car or industrial spray painters (male) exposed long-term to low levels of organic solvents (*n* = 80); 2 groups of matched controls; 80 nonexposed male industrial workers in each control group.	Long term, low level expo to multiple solvents; Assessed by interviews, on-the-job measurements, and a 1955 workshop model; Blood analysis: mean values were within normal limits for both groups; Exposed group had significantly higher values for alkaline phosphates, hemoglobin, hematocrit, and erythrocytes; early exposure TLVs in Sweden were significantly lower; solvents include TCE, TCA, methylene chloride, and others.	Self-administered psychiatric questionnaires, Eysenck's Personality Inventory, psychosocial structured interview, Comprehensive Psychopathological Rating Scale; Visual Evoked Responses; EEG; Electroneurography; Vibration Sense Threshold estimations; Neurological exam.	Calculation of z values; Pearson correlation; Multiple Regression Analysis.	Significant differences between controls and exposed in symptoms of neurasthenic syndrome, in reaction time, manual dexterity, perceptual speed, and short-term memory; no significant differences on verbal, spatial, and reasoning ability; Some differences on EEG, VER, ophthalmologic, and CT.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Gregersen, 1988	Workers exposed to organic solvents (paint, lacquer, photogravure, and polyester boat industries); Controls: warehousemen electricians; 1st follow-up 5.5 yrs after initial evaluation (59 exposed, 30 unexposed); 2nd follow-up: 10.6 yrs after initial evaluation (53 exposed, 30 unexposed controls).	1st follow-up: data about working conditions, materials and exposure in prior 5 yrs used for exposure index; 2nd follow-up: 9 questions asking about exposure to solvents in the prior 5 yrs; TCE, toluene, styrene, white spirits.	1st follow-up: structured interviews on occupational, social, medical history; clinical exam, neurological exam; 2nd follow-up: mailed questionnaire (49 follow-up issues to 1st follow-up).	Wilcoxon-Mann-Whittney tests; Kruskal-Wallis test; Chi-square; Spearman Rank Partial Correlation Coefficient.	More acute neurotoxic symptoms in exposed group at both follow-ups, but fewer symptoms at 2nd follow-up than at 1st follow-up; at both follow-ups exposed participants had more encephalopathy symptoms, especially memory and concentration; no encephalopathy symptoms in control group; symptoms and signs of peripheral, sensory, and motor neuropathy significantly worse in participants still exposed; Exposure index showed dose-effect with memory and concentration; Both follow-ups: improvement in acute symptoms; aggravation in CNS; more symptoms of peripheral nervous system and social consequences.
Juntunen et al., 1980	37 patients with suspected organic solvent poisoning (mean age = 40.1 yrs.); selection based on pneumoencephalography; no controls.	Patients were exposed to Carbon disulphide (n = 6), trichloroethylene (5), styrene (1), thinner (2), toluene (1), methanol (1), and carbon tetrachloride (2), mixtures (19); Exposure was assessed by patients' and employers' reports and measurements of air concentrations when available.	Neurologic examination, pneumoencephalographic exam, EEG, tests assessing intelligence, memory and learning, motor function, and personality.	Descriptive Statistics.	Clinical neurological findings of slight psychoorganic alterations, cerebellar dysfunction, and peripheral neuropathy; 63% had indication of brain atrophy; 23 of the 28 patients examined with electroneuromyography showed signs of peripheral neuropathy; 94% had personality changes, 80% had psychomotor deficits, 69% had impaired memory, and 57% had intelligence findings; No dose-effect found.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Juntunen et al., 1982	80 (41 women, 39 men) Finnish patients diagnosed 3−9 yrs prior with chronic solvent exposure (mean age = 38.6 yrs); 31 had slight neurological signs; no controls.	Assessed by patients' occupational history, employers' workplace description, observations and data collected at workplace, environmental measurements, biological tests; TCE, PCE, or mixed solvent exposures.	Neurologic examination; EEG and ENMG; tests of intellectual function, memory, learning, personality and psychomotor performance.	Chi-square, Maxwell-Stuart, Correlation and multiple linear regression analyses.	Significant correlations between prognosis of disturbances in gait ($p < 0.05$) and station and length of follow-up, duration and level of exposure and multiplying the two; no gender effects; Common subjective symptoms; headaches, fatigue, and memory problems; Impairment in fine motor skills, gait, and cerebellar functions; Subjective symptoms decreased during follow-up, but clinical signs increased.
Laslo-Baker et al., 2004	32 mothers with occupational exposure to organic solvents during pregnancy and their children (3−9 yrs of age); included if exposure started in 1st trimester and lasted for at least 8 wks of pregnancy (32 mother-child pairs); Controls: 32 unexposed control mothers matched on age, child age, child sex, SES, and reported cigarette use and their children (32 mother-child pairs).	Exposure information collected at 3 times: (1) during pregnancy, (2) when contacted for study participation later in pregnancy, (3) at time of assessment; Information collected included types of solvent, types of setting, duration of exposure during pregnancy, use of protection, symptoms, ventilation; Solvents include toluene (*n* = 12 women), xylene (10), ethanol (7), acetone (6), methanol (5), TCE (3), etc. (a total of 78 solvents were reported).	Children: Wechsler Preschool and Primary Scale of Intelligence, WISC, Preschool Language Scale, Clinical Evaluations of Language Fundamentals, Beery-Buktenica Developmental test of Visuo-Motor Integration, Grooved Pegboard Test, Child Behavior Checklist (Parent Version), Connor's Rating Scale-Revised (Parent Version), Behavioral Style Questionnaire; Mothers: WASI.	Power analysis, Multiple linear regression.	Verbal IQ was lower (104) in children exposed *in utero* vs. unexposed children controls (110); Children did not differ between groups in birth weight, gestational age, or developmental milestones; Children in the exposed group had significantly lower VIQ (108) and Full IQ (108) than controls (VIQ = 116 and Full IQ = 114; No significant difference in PIQ; Performance on expressive language, total language, and receptive language was significantly worse in children from exposed group.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Lee et al., 1998	40 Korean female shoe factory workers employed there for at least 5 yrs.; cases with head injury, neurological or psychological disorder, or hearing or visual impairment were excluded; Controls: 28 (housekeepers); no in-plant controls available.	4 workers wore passive personal air samplers for a full 8-h shift; Detected solvents: toluene, methyl ethyl ketone, *n*-hexane, *c*-hexane, cyclohexane, dichloroethylene, trichloroethylene, benzene, and xylene; In frame-making air concentration of solvents was 0.46−0.71; In adhesive process solvent air concentrations were 1.83−2.39; three exposure indices were calculated: current exposures, exposure duration (yrs), and Cumulative Exposure Estimate (CEE) (yrs × average exposures).	Questionnaire; Neurobehavioral Core Test Battery (includes POMS, Simple Reaction Time, Santa Ana Dexterity test, Digit Span, Benton Visual Retention Test, Pursuit aiming motor steadiness test); POMS was excluded because of cultural inapplicability.	Multivariate ANOVA for tests with 2 outcomes; ANOVA for tests with 1 outcome; education was adjusted in analyses.	Significant differences between groups based on exposure index; Differences in performance between controls and participants on Santa Ana were found only in the CEE (participants performed worse); CEE is a more sensitive measure of exposure to organic solvents.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Lindstrom, 1973	168 male workers with suspected occupational exposure to solvents Group I with solvent poisoning (*n* = 42); Group II with solvent exposure, undergoing mandatory periodic health check (*n* = 126); Control-50 healthy nonexposed male volunteers working in a viscose factory; Group IV 50 male workers with carbon disulfide poisoning.	44 exposed to TCE, 8 to tetrachloroethylene, 26 to toluene, 25 to toluene and xylene, 44 to thinners, 21 to "miscellaneous;" Solvent-exposed group had an average of 6 y of expo; CS_2 group had average of 9 yrs of exposure.	WAIS: Similarities, Picture Completion, Digit Symbol; Bourdon-Wiersma vigilance test, Santa Ana, Rorschach Inkblot test, Mira test.	Student's t-test.	The solvent-exposed group and CS_2 group had significantly worse "psychological performances" than controls; Greatest differences in sensorimotor speed and psychomotor function; solvent-exposed and CS_2 groups had deteriorated visual accuracy.
Lindstrom, 1980	56 male workers diagnosed with occupational disease caused by solvents; Controls: 98 styrene-exposed workers; 43 nonexposed construction workers.	Chronic "excessive" exposure: Mean duration of exposure = 9.1 yrs (SD = 8.3); Exposed to; halogenated and aromatic hydrocarbons, paint solvents, alcohols, and aliphatic hydrocarbons (TCE *n* = 14); Individual exposure levels estimated as time-weighted averages, based on information provided by subjects, employer, or workplace measurements, were categorized as low (3 patients), intermediate (26 patients), and high (27 patients).	WAIS subtests: Similarities, Digit Span, Digit Symbol, Picture Completion, Block Design; WMS subtests: Visual Reproduction; Benton Visual Retention test; Symmetry Drawing; Santa Ana Dexterity test; Mira test.	Factor analysis; Student's t-test; Multivariate Discriminant analysis.	Significant decline in visuomotor performance and freedom from distractibility (attention) in the solvent-exposed participants; significant relationship between duration of solvent exposure and visuomotor performance; solvent exposure level was not significant; psychological test performance of styrene-exposed control was only slightly different from nonexposed controls.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Lindstrom et al., 1982	86 Patients with prior diagnosis of solvent intoxication (mean age 38.6 yrs.); 40 male, 46 female; 52 exposed to mixed solvents; 21 exposed to TCE or PCE; 13 exposed to both; results at follow-up compared to those at initial diagnosis.	Mean duration of exposure 10.4 yrs; solvents: trichloroethylene, perchloroethylene, solvent mixture; based on patients' and/or employers' reports.	Intellectual Function: from WAIS – Similarities, Block Design, Picture Completion; Short Term Memory: from WMS – Digit Span, Logical Memory, Visual Reproduction; Benton Visual Retention test; Sensory and Motor Functions: Bourdon Wiersma Vigilance Test, Symmetry Drawing, Santa Ana Dexterity test, Mira test.	Frequency distributions, Student's t-test for paired data, stepwise linear regression.	All patients grouped together regardless of types of past solvent exposure; on follow-up, significant learning effects for Similarities when compared to results at initial diagnosis; group mean for intellectual functioning increased; no significant change in memory test results; group means for sensory and motor tasks were lower; prognosis was better for longer follow-up and younger age and poorer for users of medicines with neurological effects.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Marshall et al., 1997	All singleton births in 1983−1986 in 188 New York State counties (total number not specified); 473 CNS-defect births and 3,305 musculoskeletal-defect births; Controls: 12,436 normal births; Exclusion criteria: Trisomy 13, 18, or 21, birth weight of less than 1,000 g, sole diagnosis of hydrocephaly or microencephalopathy, hip subluxation.	Information on inactive waste sites was examined, including air vapor, air particulates, groundwater exposure via wells, and groundwater exposure. via basements; exposure was categorized as "high," "medium," "low," or unknown based on probability of exposure; proximity to waste sites was also considered; Most common solvents: TCE, toluene, xylenes, tetrachloroethene, 1,1,1-trichloroethane; Most common metals found lead, mercury, cadmium, chromium, arsenic, and nickel.		Odds ratios (OR), Fisher's exact test, Chi-square, unconditional logistic regression.	13 CNS cases and 351 controls with potential exposures; crude OR: 0.98; When controlling for mother's education, prenatal care, and exposure to a TCE facility, OR was 0.84; CNS and solvents OR: 0.8; CNS and metals OR: 1.0, musculoskeletal defects and solvents OR: 0.9, musculoskeletal defects and pesticides OR: 0.8; higher risk for CNS defects when living close to solvent-emitting facilities.
McCarthy and Jones, 1983	384 industrial workers with solvent poisoning; 103 operated degreasing baths, 62 maintained degreasing baths, 37 used TCE in portable form, 37 misc; no controls.	Individuals poisoned with trichloroethylene, perchloroethylene, and methylchloroform were examined retrospectively; Medical record review; 288 exposed to TCE, 44 to perchloroethylene, 52 to 1,1,1-trichloroethane.	Symptoms reported in occupational/medical records from industrial poisoning incidents; data from 1961 to 1980 on demographics, occupation, work process, type of industry, if incident caused fatality.		17 fatality cases, with 10 in confined spaces; Most common symptoms include effects on CNS; Gastrointestinal and Respiratory symptoms; no strong evidence for cardiac and hepatic toxicity; no change in affected number of workers in 1961 to 1980; greatest effect due to narcotic properties.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Mergler et al., 1991	54 matched pairs; Matching on the basis of age, sex, ethnicity, educational level, sex, and number of children taken from180 former microelectronics workers exposed to multiple organic solvents and control population of 157 recruited from the same region.	Average duration of employment: 6.1 yrs (range: 1−15 yrs); information about products used and chemical make-up from employer; chemicals: chlorofluorocarbons, chlorinated hydrocarbons, glycol ethers, isopropanol, acetone, toluene, xylene, and ethyl alcohol.	Sociodemographic questionnaire; Monocular examination of visual function: Far visual acuity using a Snellen chart, near visual acuity using a National Optical Visual Chart, color vision using Lanthony D-15, near contrast sensitivity using Vistech grating charts.	Signed-rank Wilcoxon test; Mann-Whitney; Chi-square test for matched pairs; Multiple Regression; Stepwise regression.	Significant difference in near contrast sensitivity: 75% of exposed workers with poorer contrast sensitivity at most frequencies than the matched controls (no difference in results based on smoking, alcohol use, and near visual acuity loss); Significant differences on near visual acuity, color vision, and rates of acquired dyschromatopsia for one eye only; No difference between groups in near or far visual acuity.
Morrow et al., 1989	22 male patients with exposure to multiple organic solvents; 4 involved in litigation; Exclusion: neurologic or psychiatric disorder prior to assessment, alcohol consumption more than 2 drinks/day; Average yrs education 12 (range: 10−16 yrs); average age 38 yrs (range: 27−61); compared to responses of WWII prisoner of war (POW) population with posttraumatic stress disorder (PTSD).	Exposure assessed with questionnaire (duration, type of solvents, weeks since last exposure, cases of excessive exposure); Average exposure duration = 7.3 yrs (range: 2 mos−19 yrs); average weeks since last exposure was 19.8 (range: 1−84 wks); 28% had at least one instance of excessive exposure.	Exposure questionnaire, Group form of the MMPI.	Stepwise multiple regression.	All profiles valid; 90% with at least 2 elevated scales above T score of 70 (clinically significant); Highest elevations on scales 1, 2, 3, and 8; only 1 case within normal limits; when compared to a group of nonpsychiatric patients, exposed patients had more elevations, although both groups have physical complaints; When compared with WWII POW (1/2 diagnosed with PTSD) with similar SES and education, both groups have similar profiles; no age effects found; significant positive correlation between scale 8 and duration of exposure; no significant difference based on time since last exposure or on experiencing excessive exposure.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Morrow et al., 1992	9 men and 3 women occupationally exposed to multiple organic solvents with CNS complaints; all met criteria for mild toxic encephalopathy; exposed group average age was 47 y; Controls: 19 (healthy male volunteers); 26 psychiatric controls (male patients with chronic schizophrenia) average age unexposed controls: 34 yrs; average age schizophrenic patients.: 36 yrs.	Exposure assessed with occupational and environmental exposure questionnaire; mean duration of expo = 3 y (range = <1 d−30 y); average time between last exposure and assessment was 2 y (range; 2 mos−10 y); solvents toluene, TCE.	Auditory event-related potentials under the oddball paradigm: counting and choice reaction time tasks.	Repeated measures ANOVA.	Exposed patients had significant delays in N250 and P300 compared to normal controls and in P300 compared to psychiatric controls; Exposed patients had higher amplitudes for N100, P200, and N250; no difference in P300 amplitude between groups; for the exposed group, P300 positively correlated with exposure duration; findings indicate that solvent exposure affects neural networks.
Seppäläinen and Antti-Poika, 1983	87 patients with solvent poisoning (40 male and 47 female) with occupational exposure to solvents; Follow-up 3−9 yrs after initial diagnosis; Mean age at diagnosis 38.6 (range: 20−59 yrs); no control population.	Chronic exposure with average duration of 10.7 yrs (range:1−33); patients were exposed to TCE (n = 21), perchloroethylene (n = 12), mixtures of solvents (n = 53), mixtures and TCE or perchloroethylene (n = 13); Exposure of 54 patients stopped after diagnosis, 33 continued to be exposed; at follow-up, only 5 working with potential of some exposure.	EEG using 10/20 system with 25−30 mins of recording, 3 mins hyperventilation and intermittent photic stimulation; ENMG.	Chi-square, Hypergeometric distribution, McNemar test.	Significantly more ENMG abnormalities at follow-up than at initial diagnosis; Most common finding: slight polyneuropathy; 43% showed improved ENMG, 33% had deteriorated, and 18 pts. with similar ENMG findings (6 normal at both exams); at follow-up, slow-wave abnormalities decreased and paroxysmal abnormalities increased; 41 with improved EEG, 28 with similar EEG (19 had normal EEG at diagnosis), and 18 with deteriorated EEG; EEG pattern of change compared to external head injuries.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Shlomo et al., 2002	Male industrial workers; Mercury exposure group (*n* = 40); average age 49.7 (±6.4) yrs; chlorinated hydrocarbons (CHs) exposure group (*n* = 37) average age 46.0 (±4.73); Controls, unexposed (*n* = 36) average age 49.8 (±5.8), matched by age; (industries not specified).	Interview and record review; Urine samples collected at end of work shift prior to testing and tested for mercury and TCA ; chlorinated hydrocarbons: TCE (*n* = 7), PCE (*n* = 8), trichloroethane (*n* = 22); Mean duration of chloral hydrate (CH) exposure 15.8 (±7.2) yrs; Mean duration of mercury exposure 15.5 (±6.4) yrs; Air sampling: mercury: 0.008 mg/m^3 (TLV = 0.025); TCE: 98 ppm (TLV = 350); PCE: 12.7 ppm (TLV = 25); trichloroethane: 14.4 ppm (TLV = 200); Blood levels: mercury (B-hg) 0.5 gr% (±0.3); TCA urine levels: 1−80% of Biologic Exposure Index (BEI); CH urine levels: 0.11−0.2 of BEI.	Medical history, Neurological tests assessing cranial nerves and cerebellar function; Otoscopy, review of archival data from pure-tone audiometric tests; Auditory brain stem responses (ABR).	Student's t-test, proportions test.	Significant differences between exposed and controls: 33.8% of CH exposed workers with abnormal IPL I-III; 18% of controls; Authors suggest ABRs are sensitive for detecting subclinical CNS effects of CH and mercury.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al., 2001	The children of mothers who had contacted a Canadian pregnancy risk counseling program during pregnancy and reported occupational exposure to solvents (*n* = 33); children age range: 3−7; Mothers' occupations: lab technicians, factory workers, graphic designers, artists, and dry cleaning; Controls: 28 matched on age, gender, parental SES, and ethnicity; children of mothers exposed to nonteratogenic agents.	Structured questionnaire about exposure; Method: weight assigned to each exposure Parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low (*n* = 19) and high (*n* = 14) exposures; solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	NEPSY: Visual Attention, Statue, Tower, Body Part Naming, Verbal Fluency, Speeded Naming, Visuomotor Precision, Imitating Hand Positions, Block Construction, Design Copying, Arrows; Peabody Picture Vocabulary Test; WRAVMA Pegboard test; Child Behavior Checklist (Parent form); Continuous Performance Test.	Mantel Haenszel test, t-test, ANCOVA, Hierarchical multiple linear regression.	Lower composite neurobehavioral scores as exposure increased after adjusting for demographics in Receptive language, expressive language, graphomotor ability; Significantly more exposed children rated with mild-severe problems; No significant difference between groups in attention, visuo-spatial ability, and fine-motor skills; Mean difference on broad- and narrow-band scales of Child Behavior Checklist scores not significant.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al., 2001	Children of mothers who had contacted a Canadian pregnancy risk counseling program during pregnancy and reported occupational exposure to solvents (*n* = 32); children age range: 3−7; Mothers' occupations: lab technicians, factory workers, graphic designers, artists, and dry cleaning; Controls: 27 matched on age, gender, parental SES, and ethnicity; children of mothers exposed to nonteratogenic agents.	Structured questionnaire about exposure; Method: weight assigned to each exposure parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low (*n* = 19) and high (*n* = 14) exposures; solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	Minimalist test to assess color vision; Cardiff Cards to assess visual acuity.	Independent samples t-tests, Mantel Haenszel Chi test; Wilcoxon-Mann-Whitney test; Kruskal-Wallis Chi square.	Significantly higher number of errors on red-green and blue-yellow discrimination in exposed children compared to controls; exposed children had poorer visual acuity than controls; No significant dose-response relationship between exposure index and color discrimination and visual acuity.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Till et al., 2005	21 infants (9 male, 12 female)of mothers who contacted a Canadian pregnancy risk counseling program and reported occupational exposure to solvents (occupations: factory, lab., dry cleaning; Controls: 27 age-matched infants (17 male, 10 female) of mothers contacted the program due to exposure during pregnancy to nonteratogenic substances).	Structured questionnaire about exposure; Method: weight assigned to each exposure parameter (length of exposure, frequency of exposure, symptoms); sum of scores for each parameter used as exposure index; median split used to categorize in low and high exposures; exposure groups: (1) aliphatic and/or aromatic hydrocarbons (*n* = 9), (2) alcohols (*n* = 3), (3) multiple solvents (*n* = 6), (4) PCE, (*n* = 3); mean duration of exposure during pregnancy 27.2 wks. (SD 7.93, range = 12−40); solvents include benzene, toluene, methane, ethane, TCE, methyl chloride, etc.	1st visit: Sweep visual evoked potentials (VEP) to assess contrast sensitivity and grating acuity; 2nd visit (2 wks after 1st): Transient VEPs to assess chromatic and achromatic mechanisms; ophthalmological exam, physical and neurological exam; testers masked to exposure status of infant.	Median split; Multiple Linear Regression; Chi-square, t-test, Mann-Whitney U test, Multivariate ANCOVA, Pearson correlation, Logistic Regression.	Significant decline of contrast sensitivity in low and intermediate spatial frequencies in exposed infants when compared with controls; Significant effect of exposure level on grating acuity, 26.3% of exposed (but 0% of controls) with abnormal VEP to red-green onset stimulus; No differences between groups in latency and amplitude of chromatic and achromatic response.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Valic et al., 1997	138 occupationally exposed and 100 unexposed controls; Exclusion criteria: congenital color vision loss, severe ocular disease, significant vision impairment, tainted glasses or contact lenses, diabetes mellitus, neurological disease, prior severe head or eye injuries, alcohol abuse, medication impairing color vision.	Solvents: TCE, PCE, toluene, xylene; Historical data on duration of exposure protective equipment use, subjective evaluation of exposure, nonoccupational solvent exposure, solvent-related symptoms at work, alcohol and smoking, drug intake; Mean urinary levels of trichloroacetic acid: 1.55 (±1.75) mg/L.	Lanthony D15.	Polytomous logistic regression.	Significant effect of age in exposed group; With alcohol of <250 g/wk no significant correlation between color confusion and solvent exposure; Significant interaction between solvent exposure and alcohol intake; Color Confusion Index significantly higher in exposed group with alcohol use of >250 g/wk.
Windham et al., 2006	Children born in 1994 in San Francisco Bay Area with Autism Spectrum Disorders (ASDs)(*n* = 284) and controls (*n* = 657), matched on basis of gender and month of birth.	Birth addresses were geocoded and linked to hazardous air pollutant database; Exposure levels assigned for 19 chemicals; chemicals were grouped based on mechanistic and structural properties; Summary index scores were calculated; risk of ASD calculated in upper quartiles of groups or individual chemical concentrations; Adjustment for demographic factors.	Archival data.	Pearson correlation, Logistic Regression.	Elevated adjusted odds ratios for ASD (by 50%) in top quartile of chlorinated solvents, but not for aromatic solvents; AOR for TCE in 4^{th} quartile = 1.47; lessened when adjusted for metals; correlation between hydrocarbon and metals exposures; when adjusted, increased risk for metals (in 3^{rd} quartile = 1.95; in 4^{th} quartile = 1.7). Contributing compounds: mercury, cadmium, nickel, TCE, vinyl chloride; Results interpreted to suggest relationship between autism and estimated metal and solvent concentrations in air around place of birth residence.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Epidemiological Studies: Controlled Exposure Studies; Neurological Effects of Trichloroethylene/Mixed Solvents					
Levy et al., 1981	9 participants (8 males and 1 female) recruited through newspaper ad; 8 h fasting before testing; no control.	Experiment 1: alcohol consumption (3 doses)—blood alcohol levels were measured with breath analyzer pre (multiple baselines) and post test (multiple). Experiment 2: Chloral hydrate administered orally over 2 mins in either 500 mg or 1,500 mg dose; multiple baseline smooth pursuit eye movement (SPEM) tests and multiple posttests after exposure; No control dose administered.	SPEM tests of following a sinusoidally oscillated target at 0.4 Hz; eye movements were recorded through electrodes at each eye.	t-tests; ANOVA.	Experiment 1: prealcohol all subjects had intact SPEM; no significant effect for 1.5 mL/kg of alcohol; significant decline in SPEM at 2.0 and 3.0 mL/kg alcohol; significant dose-effect. Experiment 2: at 500 mg. chloral hydrate, no significant change in pursuit was noted; at 1,500 mg chloral hydrate, qualitative disruptions in pursuit in all participants (4); at 500 mg participants observed to be drowsy; When number reading was added SPEM impairment was 'attenuated' in both alcohol and chloral hydrate conditions.

Table D-2. Epidemiological studies: Neurological effects of trichloroethylene/mixed solvents (continued)

Reference	Study population	Exposure assessment and biomarkers	Tests used	Statistics	Results
Stopps and McLaughlin, 1967	Chamber study using 2 healthy male volunteers exposed to Freon-113; 1 volunteer exposed to TCE; No control.	Exposure booth was constructed; TCE in air: TCE concentrations: 100, 200, 300, 400 ppm (1965 TLV: 100 ppm for 8-h exposure) in ascending and descending order; total time in chamber: 2.75 h; Freon-113 concentrations: 1,500, 2,500, 3,500, 4,500 ppm (1965 TLV: 1,000 ppm for 8-h exposure), duration 1.5 h; TCE: (1) reduction of weight of compound during exposure was calculated, (2) continuous air sampling in the chamber; Freon-113 in air: (1) and (2) same; (3) gas chromatography on air captured in bottles sealed in the chamber; no control dose given.	Crawford Small Parts Dexterity Test, Necker Cube Test, Card Sorting, Card Sorting with an Auxiliary Task, Dial Display (TCE participant only); Short Employment Test-Clerical (Freon-113 participants only).	Descriptive statistics for air measurement plots by % of TCE change in groups.	No TCE effect at 100 ppm, but test performance deteriorated with increase of TCE concentration; No effect of Freon-113 on psychomotor function at 1,500 ppm, deterioration at 2,500 ppm, as concentration increased, performance deteriorated.

CNS = central nervous system, EEG = electroencephalograph, PCE = perchloroethylene, WHO = World Health Organization.

Table D-3. Literature review of studies of TCE and domains assessed with neurobehavioral/neurological methods

Authors	Year	Study type	Participants no. (N = exposed C = nonexposed)	Dur	PM/RT	VM	Cogn	M&L	M&P	Symp†	Sen††	Resp	Dose effect √√ urinary metabolites√	TCE levels
ATSDR	2003	E	N = 116, C = 177	**C**	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	**A**	*ne*	*ne*	0 → 23 ppb in dg water
Barret et al.	1984	O	N = 188	C	*ne*	*ne*	*ne*	*ne*	*ne*	H, D	T, N, V	*ne*	√	150 ppm
Barret et al.	1987	O	N = 104, C = 52	C	*ne*	*ne*	*ne*	*ne*	√	H, D, S, I	T, N	*ne*	√	*ne*
Barrett, et al.	1982	O	N = 11, C = 2	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T	*ne*	√	*ne*
Burg, et al.	1995	E	N = 4,281	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	A, N	√	√	*ne*
Burg and Gist	1999	E	N = 3915	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	A, N	√	√√	4 gps: 2−75,000 ppb
El Ghawabi et al.	1973	O	N = 30, C = 30	C	*ne*	*ne*	*ne*	*ne*	*ne*	H, S	(-)	*ne*	√	165 ppm
Feldman et al.	1988	E	N = 21, C = 27	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T	*ne*	*ne*	*ne*
Feldman et al.	1992	O	N = 18, C = 30	A,C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T, N	*ne*	*ne*	*ne*
Gamberale, et al.	1976	C	N = 15	A	√	*ne*	√	(-)	*ne*	*ne*	*ne*	*ne*	*ne*	540−1,080 mg^3
Gash et al.	2007	O	N = 30	C	√	*ne*	*ne*	*ne*	*ne*	M, N		*ne*	*ne*	*ne*
Grandjean et al.	1955	O	N = 80	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√, √√	6−1,120 ppm
Gun, et al.	1978	O	N = 8, C = 8	C	√	*ne*	√	*ne*	*ne*	*ne*	N	*ne*	*ne*	3−418 ppm
Hirsch, et al.	1996	E	N = 106	C	*ne*	*ne*	*ne*	*ne*	*ne*	H	*ne*	*ne*	*ne*	0−2,441 ppb
Kilburn and Thornton	1996	E	N = 237, C = 264	C	√	*ne*	√	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*
Kilburn and Warshaw	1993	E	N = 544, C = 181	C	√	√	√	√	√	M	T, N	*ne*	*ne*	6−500 ppb
Kilburn	2002a	E	N = 236, C = 228	C	*ne*	*ne*	√	*ne*	*ne*	M	B	*ne*	*ne*	6−500 ppb
Kilburn	2002b	E	N = 236, C = 58	C	(-)	*ne*	*ne*	*ne*	(-)	*ne*	*ne*	*ne*	*ne*	0.2−1,000 ppb
Konietzko, et al.	1975	C	N = 20	A	*ne*	*ne*	*ne*	*ne*	*ne*	M	N	*ne*	√	953 ppm
Kylin, et al. 1967	1967	C	N = 12	A	√	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	*ne*	1,000 ppm
Landrigan, et al.	1987	O	Residents and 12 W	A,C	*ne*	*ne*	√	*ne*	*ne*	H, D	*ne*	*ne*	√√	≥183,000 ppb
Liu, et al.	1988	O	N = 103, C = 111	C	*ne*	*ne*	*ne*	√	*ne*	D, N	N	*ne*	√√	1−100 ppm
Mhiri et al.	2004	O	N = 23, C = 23	A	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	T	*ne*	√, √√	*ne*
Nagaya et al.	1990	O	N = 84, C = 83	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√	22 ppm

Table D-3. Literature review of studies of TCE and domains assessed with neurobehavioral/neurological methods (continued)

Authors	Year	Study type	**Participants no.** (N = exposed C = nonexposed)	Dur	PM/RT	VM	Cogn	M&L	M&P	Symp†	Sen††	Resp	Dose effect √√ urinary metabolites√	TCE levels
Rasmussen and Sabroe	1986	O	N = 240, C = 350	C	*ne*	*ne*	*ne*		√	H,D, I, M	*ne*	*ne*	*ne*	*ne*
Rasmussen et al.	1993	O	N = 96	C	*ne*	*ne*	√	*ne*	*ne*	*ne*	*ne*	*ne*	√√	*ne*
Rasmussen et al.	1993	O	N = 96	C	*ne*	√	√	*ne*	*ne*	*ne*	*ne*	*ne*	√√	*ne*
Rasmussen et al.	1993	O	N = 99	C	√	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√√	*ne*
Reif et al.	2003	E	N = 143	C	√	√	*ne*	*ne*	√	M	M	*ne*	√√	5−15 ppb
Ruijten, et al.	1991	O	N = 31, C = 28	C	√	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	17−70 ppm
Smith	1970	O	N = 130, C = 63	C	*ne*	*ne*	*ne*	*ne*	*ne*	H, D	**N**	*ne*	√, √√	*ne*
Stewart et al	1970	C	N = 13	A	*ne*	*ne*	√	*ne*	*ne*	H	*ne*	*ne*	√	100−202 ppm
Triebig, et al.	1976	C	N = 7, C = 7	A	*ne*	*ne*	√	√	√	(-)	*ne*	*ne*	√, √√	0−100 ppm
Triebig, et al.	1977	C	N = 7, C = 7	A	*ne*	*ne*	√	√	√	M	(-)	*ne*	√, √√	0−100 ppm
Triebig, et al.	1977	O	N = 8	A,C	*ne*	√	√	√	*ne*	*ne*	*ne*	*ne*	√	50 ppm
Triebig, et al.	1982	O	N = 24, C = 24	C	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	N	*ne*	√, √√	5−70 ppm
Triebig, et al.	1983	O	N = 66, C = 66	C	*ne*	*ne*	*ne*	*ne*	*ne*	N, H	N	*ne*	√	10−600 mg/m^3
Troster and Ruff	1990	O	N = 3, C = 60	A	√	√	√	√	√	*ne*	N	*ne*	*ne*	*ne*
Vernon and Ferguson	1969	C	N = 8	A	√	√	*ne*	*ne*	*ne*	*ne*	N	*ne*	√√	0−1000 ppm
Windemuller and Ettema	1978	C	N = 39	A	√	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	200 ppm
Winneke	1982	O	Not reported	*ne*	(-)	(-)	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	*ne*	50 ppm

†**H** = Headaches; **D** = Dizziness; **I** = Insomnia; **S** = Sex Probls; **M** = Mood; **N** = Neurological.
††**A** = Audition; **B** = Balance; **V** = Vision; **T** = Trigeminal nerve; **N** = Other Neurological.

Study: **C** = Chamber; **E** = Environmental; **O** = Occupational.
Duration: **A** = Acute, **C** = Chronic.

√ = positive findings; (-) = findings not significant; *ne* = not examined or reported; Dur = duration; PM/RT = psychomotor/reaction time; VM = visuo-motor; Cogn = cognitive; M&L = memory and learning; M&P = mood and personality; Symp = symptoms; Sen = sensory; Resp = respiratory.

D.2. CENTRAL NERVOUS TOXICITY IN ANIMAL STUDIES FOLLOWING TRICHLOROETHYLENE (TCE) EXPOSURE

In vivo studies in animals and *in vitro* models have convincingly demonstrated that TCE produces functional and physiological neurological changes. Overall, these effects collectively indicate that TCE has central nervous system (CNS) depressant-like effects at lower exposures and causes anesthetic-like effects at high exposures. Studies of TCE toxicity in animals have generally not evaluated whether or not adverse effects seen acutely persist following exposure or whether there are permanent effects of exposure. Exceptions to the focus on acute impairment while under TCE intoxication include studies of hearing impairment and histopathological investigations focused primarily on specific neurochemical pathways, hippocampal development, and demyelination. These persistent TCE effects are discussed initially followed by the results of studies that examined the acute effects of this agent. Summary tables for all the animal studies are at the end of this section.

D.2.1. Alterations in Nerve Conduction

There is little evidence that TCE disrupts trigeminal nerve function in animal studies. Two studies demonstrated TCE produces morphological changes in the trigeminal nerve at a dose of 2,500 mg/kg-day for 10 weeks (Barret et al., 1991, 1992). However, dichloroacetylene, a degradation product formed during the volatilization of TCE was found to produce more severe morphological changes in the trigeminal nerve and at a lower dose of 17 mg/kg-day (Barret et al., 1991, 1992). Only one study (Albee et al., 2006) has evaluated the effects of TCE on trigeminal nerve function, and a subchronic inhalation exposure did not result in any significant functional changes. A summary of these studies is provided in Table D-4.

Barret et al. (1991, 1992) conducted two studies evaluating the effects of both TCE and dichloroacetylene on trigeminal nerve fiber diameter and internodal length as well as several markers for fiber myelination. Female Sprague Dawley rats (n = 7/group) were dosed with 2,500 mg/kg TCE or 17 mg/kg-day dichloroacetylene by gavage for 5 days/week for 10 weeks. These doses were selected based upon the ratio of the LD_{50}s (dose at which there is 50% lethality) for these two agents. Two days after administration of the last dose, a morphometric approach was used to study the diameter of teased fibers from the trigeminal nerve. The fibers were classified as Class A or Class B and evaluated for internode length and fiber diameter. TCE-dosed animals only exhibited changes in the smaller Class A fibers where internode length increased marginally (<2%) and fiber diameter increased by 6%. Conversely, dichloroacetylene-treated rats exhibited significant and more robust decreases in internode length and fiber diameter in both fiber classes A and B. Internode length decreased 8% in Class A fibers and 4% in Class B fibers. Fiber diameter decreased 10% in Class A fibers and 6% in Class B fibers.

Biochemical data are presented for fatty acid composition from total lipid extractions from the trigeminal nerve. These two studies identify a clear effect of dichloroacetylene on trigeminal nerve fibers, but the effect by TCE is quite limited.

Albee et al. (2006) evaluated the effects of a subchronic inhalation TCE exposure in Fischer 344 rats (10/sex/group). Rats were exposed to 0-, 250-, 800-, and 2,500-ppm TCE for 6 hours/day, 5 days/week for 13 weeks. At the eleventh week of exposure, rats were surgically implanted with epidural electrodes over the somatosensory and cerebellar regions, and TSEPs were collected 2−3 days following the last exposure. TSEPs were generated using subcutaneous needle electrodes to stimulate the vibrissal pad (area above the nose). The resulting TSEP was measured with electrode previously implanted over the somatosensory region. The TCE exposures were adequate to produce permanent auditory impairment even though TSEPs were unaffected. While TCE appears to be negative in disrupting the trigeminal nerve, the TCE breakdown product, dichloroacetylene, does impair trigeminal nerve function.

Albee et al. (1997) reported that dichloroacetylene disrupted trigeminal nerve somatosensory evoked potentials in Fischer 344 male rats. The subjects were exposed to a mixture of 300-ppm dichloroacetylene, 900-ppm acetylene, and 170-ppm TCE for a single 2.25-hour period. This dichloroacetylene was generated by decomposing TCE in the presence of potassium hydroxide and stabilizing with acetylene. A second treatment group was exposed to a 175-ppm TCE/1,030-ppm acetylene mix with no potassium hydroxide present. Therefore, no dichloroacetylene was present in the second treatment group, providing an opportunity to determine the effects on the trigeminal nerve somatosensory evoked potential in the absence of dichloroacetylene. Evoked potentials from the dichloroacetylene/TCE/acetylene-exposed rats were about 17% smaller measured between peaks I and II and 0.13 msec slower in comparison to the preexposure measurements. Neither latency nor amplitude of this potential changed significantly between the preexposure and postexposure test in the air-exposed animals (control). The dichloroacetylene-mediated evoked potential changes persisted at least until Day 4 postexposure. No changes in evoked potentials were observed in the 175-ppm TCE/1,030-ppm acetylene mix group. It is noteworthy that dichloroacetylene treatment produced broader evidence of toxicity as witnessed by a persistent drop in body weight among subjects over the 7-day postexposure measuring period. In light of the differences observed between the effects of TCE and dichloroacetylene on the trigeminal nerve, it would be instructive to calculate the dose of TCE that would be necessary to produce comparable tissue levels of dichloroacetylene produced in the Albee et al. (1997) study.

Kulig (1987) also measured peripheral (caudal nerve) nerve conduction time in male Wistar rats and failed to show an effect of TCE with exposures as high as 1,500 ppm for 16 hours/day, 5 days/week for 18 weeks.

D.2.2. Auditory Effects

D.2.2.1. *Inhalation*

The ability of TCE to disrupt auditory function and produce inner ear histopathology abnormalities has been demonstrated in several studies using a variety of test methods. Two different laboratories have identified NOAELs for auditory function of 1,600 ppm following inhalation exposure for 12 hours/day for 13 weeks in Long Evans rats (*n* = 6−10) (Rebert et al., 1991) and 1,500 ppm in Wistar-derived rats (*n* = 12) exposed by inhalation for 18 hours/day, 5 days/week for 3 weeks (Jaspers et al., 1993). The LOAELs identified in these and similar studies are 2,500−4,000-ppm TCE for periods of exposure ranging from 4 hours/day for 5 days to 12 hours/day for 13 weeks (e.g., Muijser et al., 2000; Rebert et al., 1995, 1993; Crofton et al., 1994; Crofton and Zhao, 1997; Fechter et al., 1998; Boyes et al., 2000; Albee et al., 2006). Rebert et al. (1993) estimated acute blood TCE levels associated with permanent hearing impairment at 125 μg/mL by methods that probably underestimated blood TCE values (rats were anaesthetized using 60% carbon dioxide). A summary of these studies is presented in Table D-5.

Rebert et al. (1991) evaluated auditory function in male Long Evans rats (*n* = 10) and F344 rats (*n* = 4−5) by measuring brainstem auditory-evoked responses (BAERs) following stimulation with 4-, 8-, and 16-kHz sounds. The Long-Evans rats were exposed to 0-, 1,600-, or 3,200-ppm TCE, 12 hour/day for 12 weeks and the F344 rats were exposed to 0-, 2,000-, or 3,200-ppm TCE, 12 hours/day for 3 weeks. BAERs were measured every 3 weeks during the exposure and then for an additional 6 weeks following the end of exposure. For the F344 rats, both TCE exposure (2,000 and 3,200 ppm) significantly decreased BAER amplitudes at all frequencies tested. In comparison, Long Evans rats exposed to 3,200-ppm TCE also had significantly decreased BAER amplitude, but exposure to 1,600 ppm did not significantly affect BAERs at any stimulus frequency. These data suggest a LOAEL at 2,000 ppm for the F344 rats and a NOAEL at 1,600 ppm for the Long Evans rats. In subsequent studies, Rebert et al. (1993, 1995) again demonstrated TCE significantly decreases BAER amplitudes and significantly increases the latency of the initial peak (identified as P1).

Jaspers et al. (1993) exposed Wistar-derived WAG-Rii/MBL rats (*n* = 12) to 0, 1,500 and 3,000-ppm TCE exposure for 18 hours/day, 5 days/week for 3 weeks. Auditory function for each frequency was assessed by reflex modification (recording the decibel threshold required to generate a startle response from the rat). Three tones (5, 20, and 35 kHz) were used to test auditory function. The startle measurements were made prior to exposure and at 1, 3, 5, and 6 weeks after exposure. A selective impairment of auditory threshold for animals exposed to 3,000-ppm TCE was observed at all postexposure times at 20 kHz only. No significant effects were noted in rats exposed to 1,500-ppm TCE. This auditory impairment was persistent up through 6 weeks after exposure, which was the last time point presented. There was no impairment of hearing at either 5 or 25 kHz for animals exposed to 1,500- or 3,000-ppm TCE. This study indicates TCE selectively produces a persistent mid-frequency hearing loss and identifies a NOAEL of 1,500 ppm. Similarly, Crofton et al. (1994) exposed male Long Evans rats (*n* = 7−8) to 3,500-ppm TCE, 8 hours/day for 5 days. Auditory thresholds were determined by reflex modification audiometry 5−8 weeks after exposure. TCE produced a selective impairment of auditory threshold for mid frequency tones, 8 and 16 kHz.

Muijser et al. (2000) evaluated the ability of TCE to potentiate the damaging effect of noise on hearing. Wistar rats (*n* = 8 per group) were exposed by inhalation to 0 or 3,000-ppm TCE alone for 18 hours/day, 5 days/week for 3 weeks (no noise) or in conjunction with 95-dB broad band noise. The duration of noise exposure is not specified, but presumably was also 18 hours/day, 5 days/week for 3 weeks. Pure tone auditory thresholds were determined using reflex modification audiometry 1 and 2 weeks following the exposures. Significant losses in auditory sensitivity were observed for rats exposed to noise alone at 8, 16, and 20 kHz, for rats exposed to TCE alone at 4, 8, 16, and 20 kHz and for combined exposure subjects at 4, 8, 16, 20, and 24 kHz. The loss of hearing sensitivity at 4 kHz is particularly striking for the combined exposure rats, suggesting a potentiation effect at this frequency. Impairment on this auditory test suggests toxicity at the level of the cochlea or brainstem.

Fechter et al. (1998) exposed Long Evans rats inhalationally to 0 or 4,000-ppm TCE 6 hours/day for 5 days. Three weeks later auditory thresholds were assessed by reflex modification audiometry (*n* = 12), and then 5−7 weeks later, cochlear function was assessed by measuring compound action potentials (CAPs) and the cochlear microphonic response (*n* = 3−10). Cochlear histopathology was assessed at 5−7 weeks (*n* = 4) using light microscopy. Reflex modification thresholds were significantly elevated at 8 and 18 kHz, as were CAP thresholds. The growth of the N1 evoked potential was reduced in the TCE group, and they failed to show normal N1 amplitudes even at supra-threshold tone levels. There was no effect on the sound level required to elicit a cochlear microphonic response of 1 μV. Histological data suggest that TCE produces a loss of spiral ganglion cells.

Albee et al. (2006) exposed male and female F344 rats to TCE at 250, 800, or 2,500 ppm for 6 hours/day, 5 days/week, for 13 weeks. At 2,500-ppm TCE, mild frequency-specific hearing deficits were observed, including elevated tone-pip auditory brainstem response thresholds. Focal loss of hair cells in the upper basal turn of the cochlea was observed in 2,500-ppm-exposed rats; this was apparently based upon midmodiolar sections, which lack power in quantification of hair cell death. Except for the cochleas of 2,500-ppm-exposed rats, no treatment-related lesions were noted during the neuro-histopathologic examination. The NOAEL for this study was 800 ppm based on ototoxicity at 2,500 ppm.

The relationship between dose and duration of exposure with respect to producing permanent auditory impairment was presented in Crofton and Zhao (1997) and again in Boyes et al. (2000). The LOAELs identified in Long Evans rats (*n* = 10−12) were 6,000 ppm for a 1-day exposure, 3,200 ppm per day for both the 1- and 4-week exposures, and 2,400 ppm per day for the 13-week exposure. It was estimated from these data that the LOAEL for a 2-year long exposure would be 2,100 ppm. Auditory thresholds were determined for a 16-kHz tone 3−5 weeks after exposure using reflex modification audiometry. Results replicated previous findings of a hearing loss at 16 kHz for all exposure durations. One other conclusion reached by this study is that TCE concentration and not concentration × duration of exposure is a better predictor of auditory toxicity. That is, the notion that total exposure represented by the function, concentration (C) × time (t), or Haber's law, is not supported. Therefore, higher exposure concentrations for short durations are more likely to produce auditory impairment than are lower concentrations for more protracted durations when total dosage is equated. Thus, consideration needs to be given not only to total C × t, but also to peak TCE concentration.

Crofton and Zhao (1997) also presented a benchmark dose for which the calculated dose of TCE would yield a 15-dB loss in auditory threshold. This benchmark response was selected because a 15-dB threshold shift represents a significant loss in threshold sensitivity for humans. The benchmark concentrations for a 15-dB threshold shift are 5,223 ppm for 1 day, 2,108 ppm for 5 days, 1,418 ppm for 20 days, and 1,707 ppm for 65 days of exposure. While more sensitive test methods might be used and other definitions of a benchmark effect chosen with a strong rationale, these data provide useful guidance for exposure concentrations that do yield hearing loss in rats.

These data demonstrate that the ototoxicity of TCE was less than that predicted by a strict concentration × time relationship. These data also demonstrate that simple models of extrapolation (i.e., $C \times t = k$, Haber's Law) overestimate the potency of TCE when extrapolating from short-duration to longer-duration exposures. Furthermore, these data suggest that, relative to ambient or occupational exposures, the ototoxicity of TCE in the rat is a high-concentration effect; however, the selection of a 15-dB threshold for detecting auditory impairment along with tests at a single auditory frequency may not capture the most sensitive reliable measure of hearing impairment.

With the exception of a single study performed in the Hartley guinea pig (*n* = 9−10; Yamamura et al., 1983), there are no data in other laboratory animals related to TCE-induced ototoxicity. Yamamura et al. (1983) exposed Hartley guinea pigs to TCE at doses of 6,000, 12,000, and 17,000 ppm for 4 hours/day for 5 days and failed to show an acute impairment of auditory function. However, despite the negative finding in this study, it should be considered that auditory testing was performed in the middle of a laboratory and not in an audiometric sound attenuating chamber. The influence of extraneous and uncontrolled noise on cochlear electrophysiology is marked and assesses auditory detection thresholds in such an environment unrealistic. Although the study has deficiencies, it is important to note that the guinea pig has been reported to be far less sensitive than the rat to the effects of ototoxic aromatic hydrocarbons such as toluene.

It may be helpful to recognize that the effects of TCE on auditory function in rats are quite comparable to the effects of styrene (e.g., Pryor et al., 1987; Crofton et al., 1994; Campo et al., 2006), toluene (e.g., Pryor et al., 1983; Campo et al., 1999) ethylbenzene (e.g., Cappaert et al., 1999, 2000; Fechter et al., 2007), and *p*-xylene (e.g., Pryor et al., 1987; Gagnaire et al., 2001). All of these aromatic hydrocarbons produce reliable impairment at the peripheral auditory apparatus (inner ear), and this impairment is associated with death of sensory receptor cells, the outer hair cells. In comparing potency of these various agents to produce hearing loss, it appears that TCE is approximately equipotent to toluene and less potent than, in order, ethylbenzene, *p*-xylene, and styrene. Occupational epidemiological studies do appear to identify auditory impairments in workers who are exposed to styrene (Sliwinska-Kowalska et al., 1999; Morioka et al., 2000; Morata et al., 2002) and those exposed to toluene (Abbate et al., 1993; Morata et al., 1997), particularly when noise is also present.

D.2.2.2. *Oral and Injection Studies*

No experiments were identified in which auditory function was assessed following TCE administration by either oral or injection routes.

D.2.3. Vestibular System Studies

The effect of TCE on vestibular function was evaluated by either (1) promoting nystagmus (vestibular system dysfunction) and comparing the level of effort required to achieve nystagmus in the presence and absence of TCE or (2) using an elevated beam apparatus and measuring the balance. Overall, it was found that TCE disrupts vestibular function as presented below. Summary of these studies is found in Table D-6.

Tham et al. (1979, 1984) demonstrated disruption in the stimulated vestibular system in rabbits and Sprague Dawley rats during intravenous (i.v.) infusion with TCE. It is difficult to determine the dosage of TCE necessary to yield acute impairment of vestibular function since testing was performed under continuing infusion of a lipid emulsion containing TCE, and therefore, blood TCE levels were increasing during the course of the study. Tham et al. (1979), for example, infused TCE at doses of 1−5 mg/kg/min reaching arterial blood concentrations as high as 100 ppm. They noted increasing numbers of rabbits experiencing positional nystagmus as blood TCE levels increased. The most sensitive rabbit showed nystagmus at a blood TCE concentration of about 25 ppm. Similarly, the Sprague Dawley rats also experienced increased nystagmus with a threshold effect level of 120 ppm as measured in arterial blood (Tham et al., 1984). Animals demonstrated a complete recovery in vestibular function when evaluated for nystagmus within 5−10 minutes after the i.v. infusion was stopped.

Niklasson et al. (1993) showed acute impairment of vestibular function in male and female pigmented rats during acute inhalation exposure to TCE (2,700−7,200 ppm) and to trichloroethane (500−2,000 ppm). Both of these agents were able to promote nystagmus during optokinetic stimulation in a dose related manner. While there were no tests performed to assess persistence of these effects, Tham et al. (1979, 1984) did find complete recovery of vestibular function in rabbits (n = 19) and female Sprague-Dawley rats (n = 11) within minutes of terminating a direct arterial infusion with TCE solution.

The finding that trichloroethylene can yield transient abnormalities in vestibular function is not unique. Similar impairments have been shown for toluene, styrene, along with trichloroethane (Niklasson et al., 1993) and by Tham et al. (1984) for a broad range of aromatic hydrocarbons. The concentration of TCE in blood at which effects were observed for TCE (0.9 mM/L) was quite close to that observed for most of these other vestibulo-active solvents.

D.2.4. Visual Effects

Changes in visual function have also been demonstrated in animal studies following acute (Boyes et al., 2003, 2005) and subchronic exposure (Blain et al., 1994). Summary of all TCE studies evaluating visual effects in animals can be found in Table D-6. In these studies, the effect of TCE on visual-evoked responses to patterns (Boyes et al., 2003, 2005; Rebert et al., 1991) or a flash stimulus (Rebert et al., 1991; Blain et al., 1994) were evaluated. Overall, the studies demonstrated that exposure to TCE results in significant changes in the visual evoked response, which is reversible once TCE exposure is stopped. Only one study (Rebert et al., 1991) did not demonstrate changes in visual system function with a subchronic TCE exposure, but visual testing was conducted 10 hours after each exposure.

Boyes et al. (2003, 2005) found significant reduction in the visual evoked potential acutely while Long Evans male rats were being exposed to TCE concentrations of 500, 1,000, 2,000, 3,000, 4,000, and 5,000 ppm for intervals ranging from 4 to 0.5 hours, respectively. In both instances, the degree of effect correlated more with brain TCE concentrations than with duration of exposure.

Boyes et al. (2003) exposed adult, male Long-Evans rats to TCE in a head-only exposure chamber while pattern onset/offset visual evoked potentials (VEPs) were recorded. Exposure conditions were designed to provide C × t products of 0 ppm/hour (0 ppm for 4 hours) or 4,000 ppm/hour created through four exposure scenarios: 1,000 ppm for 4 hours; 2,000 ppm for

2 hours; 3,000 ppm for 1.3 hours; or 4,000 ppm for 1 hour (n = 9−10/concentration). Blood TCE concentrations were assessed by GC with ECD, and brain TCE concentrations were estimated using a physiologically based pharmacokinetic (PBPK) model. The amplitude of the VEP frequency double component (F2) was decreased significantly ($p < 0.05$) by exposure. The mean amplitude (±SEM in μV) of the F2 component in the control and treatment groups measured 4.4 ± 0.5 (0 ppm/4 hours), 3.1 ± 0.5 (1,000 ppm/4 hours), 3.1 ± 0.4 (2,000 ppm/2 hours), 2.3 ± 0.3 (3,000 ppm/1.3 hours), and 1.9 ± 0.4 (4,000 ppm/1 hour). A PBPK model was used to estimate the concentrations of TCE in the brain achieved during each exposure condition. The F2 amplitude of the VEP decreased monotonically as a function of the estimated peak brain concentration but was not related to the area under the curve of the brain TCE concentration. These results indicate that an estimate of the brain TCE concentration at the time of VEP testing predicted the effects of TCE across exposure concentrations and duration.

In a follow-up study, Boyes et al. (2005) exposed Long Evans male rats (n = 8−10/concentration) to TCE exposures of 500 ppm for 4 hours, 1,000 ppm for 4 hours, 2,000 ppm for 2 hours, 3,000 ppm for 1.3 hours, 4,000 ppm for 1 hour and 5,000 ppm for 0.8 hour. VEP recordings were made at multiple time points, and their amplitudes were adjusted in proportion to baseline VEP data for each subject. VEP amplitudes were depressed by TCE exposure during the course of TCE exposure. The degree of VEP depression showed a high correlation with the estimated brain TCE concentration for all levels of atmospheric TCE exposure.

This transient effect of TCE on the peripheral visual system has also been reported by Blain (1994) in which New Zealand albino rabbits were exposed by inhalation to 350- and 700-ppm TCE 4 hours/day, 4 days/week for 12 weeks. Electroretinograms (ERGs) and oscillatory potentials (OPs) were recorded weekly under mesopic conditions. Recordings from the 350- and 700-ppm exposed groups showed a significant increase in the amplitude of the a- and b-waves (ERG). The increase in the a-wave was dose related increasing 30% at the low dose and 84% in the high dose. For the b-wave, the lower exposure dose yielded a larger change from baseline (52%) than did the high dose (33%). The amplitude of the OPs was significantly decreased at 350 ppm (57%) and increased at 700 ppm (117%). The decrease in the oscillatory potentials (OPs) shown in the low-dose group appears to be approximately 25% from 9−12 weeks of exposure. These electroretinal changes were reversed to the baseline value within 6 weeks after the inhalation stopped.

Rebert et al. (1991) evaluated visual evoked potentials (flash evoked potentials and pattern reversal evoked potentials) in male Long Evans rats that received 1,600- or 3,200-ppm TCE for 3 weeks 12 hours/day. No significant changes in flash evoked potential measurements were reported following this exposure paradigm. Limited shifts in pattern reversal visual evoked potentials were reported during subchronic exposure, namely a reduction in the N1-P1 response amplitude that reached statistical significance following 8, 11, and 14 weeks of exposure. The drop in response amplitude ranged from approximately 20% after 8 weeks to nearly 50% at Week 14. However, this potential recovered completely during the recovery period.

D.2.5. Cognitive Function

There have been a number of reports (e.g., Kjellstrand et al.,1980; Kulig, 1987; Kishi et al., 1993) showing alteration in performance in learning tasks such as a change in speed to complete the task, but little evidence that learning and memory function are themselves impaired by exposure. Table D-7 presents the study summaries for animal studies evaluating cognitive effects following TCE exposure. Such data are important in efforts to evaluate the functional significance of decreases in myelinated fibers in the hippocampus reported by Isaacson et al. (1990) and disruption of long-term potentiation discovered through *in vitro* testing (Ohta et al., 2001) since the hippocampus has been closely tied to memory formation.

Kjellstrand et al. (1980) exposed Mongolian gerbils (n = 12/sex**)** to 900-ppm TCE by inhalation for 9 months. Inhalation was continuous except for 1−2 hours/week for cage cleaning. Spatial memory was tested using the radial arm maze task. In this task, the gerbils had to visit each arm of the maze and remember which arm was visited and unvisited in selecting an arm to visit. The gerbils received training and testing in a radial arm maze starting after 2 months of TCE exposure. There was no effect of TCE on learning or performance on the radial arm maze task.

Kishi et al. (1993) acutely exposed Wistar rats to TCE at concentrations of 250, 500, 1,000, 2,000, and 4,000 ppm for 4 hours. Rats were tested on an active (light) signaled shock avoidance operant response. Rats exposed to 250-ppm TCE showed a significant decrease both in the total number of lever presses and in avoidance responses at 140 minutes of exposure compared with controls. The rats did not recover their pre-exposure performance until 140 minutes after the exhaustion of TCE vapor. Exposures in the range 250- to 2,000-ppm TCE for 4 hours produced concentration related decreases in the avoidance response rate. No apparent acceleration of the reaction time was seen during exposure to 1,000- or 2,000-ppm TCE. The latency to a light signal was somewhat prolonged during the exposure to 2,000- to 4,000-ppm TCE. It is estimated that there was depression of the central nervous system with slight performance decrements and the corresponding blood concentration was 40 μg/mL during exposure. Depression of the central nervous system with anesthetic performance decrements was produced by a blood TCE concentration of about 100 μg/mL. In general, they observed dose related reductions in total number of lever presses, but these changes may be more indicative of impaired motor performance than of cognitive impairment. In any event, recovery occurred rapidly once TCE exposure ceased.

Isaacson et al. (1990) studied the effects of oral TCE exposure in weanling rats at exposure doses of 5.5 mg/day for 4 weeks, followed by an additional 2 weeks of exposure at 8.5 mg/day. No significant changes were observed in locomotor activity in comparison to the control animals. This group actually reported improved performance on a Morris swim test of spatial learning as reflected in a decrease in latency to find the platform from 14 seconds in control subjects to 12 seconds in the lower dose TCE group to a latency of 9 seconds in the higher TCE group. The high dose TCE group differed significantly from the control and low TCE dose groups while these latter two groups did not differ significantly from each other. This improvement relative to the control subjects occurred despite a loss in hippocampal myelination, which approached 8% and was shown to be significant using Duncan's multiple range test.

Likewise, Umezu et al. (1997) exposed ICR strain male mice acutely to doses of TCE ranging from 62.5−1,000 mg/kg depending upon the task. They reported a depressed rate of operant responding in a conditioned avoidance task that reached significance with intraperitoneal (i.p.) injections of 1,000 mg/kg. Increased responding during the signaled avoidance period at lower doses (250 and 500 mg/kg) suggests an impairment in ability to inhibit responding or failure to attend to the signal. However, all testing was performed under TCE intoxication.

D.2.6. Psychomotor Effects

Changes in psychomotor activity such as loss of righting reflex, functional observational battery changes, and locomotor activity have been demonstrated in animals following exposure to TCE. Summaries for some of these studies can be found below and are presented in detail in Table D-8.

D.2.6.1. *Loss of Righting Reflex*

Kishi et al. (1993) evaluated the activity and performance of male Wistar rats in a series of tasks following an acute 4-hour exposure to 250, 500, 1,000, 2,000, and 4,000 ppm. They reported disruption in performance at the highest test levels with CNS depression and anesthetic performance decrements. Blood TCE concentrations were about 100 μg/mL in Wistar rats (such blood TCE concentrations were obtained at inhalation exposure levels of 2,000 ppm).

Umezu et al. (1997) studied disruption of the righting reflex following acute injection of 250, 500, 1,000, 2,000, 4000, and 5,000 mg/kg TCE in male ICR mice. At 2,000 mg/kg, loss of righting reflex (LORR) was observed in only 2/10 animals injected. At 4,000 mg/kg, 9/10 animals experienced LORR, and 100% of the animals experienced LORR at 5,000 mg/kg. Shih et al. (2001) reported impaired righting reflexes at exposure doses of 5,000 mg/kg in male

Mf1 mic although lower exposure doses were not included. They showed, in addition, that pretreatment prior to TCE with DMSO or disulfiram (which is a CYP2E1 inhibitor) in DMSO could delay loss of the righting reflex in a dose related manner. By contrast, the alcohol dehydrogenase inhibitor, 4-metylpyradine did not delay loss of the righting reflex that resulted from 5,000 mg/kg TCE. These data suggest that the anesthetic properties of TCE involve its oxidation via CYP2E1 to an active metabolite, a finding that is consistent with the anesthetic properties of chloral hydrate.

D.2.6.2. *Functional Observational Battery (FOB) and Locomotor Activity Studies*

D.2.6.2.1. *Functional observational battery (FOB) and locomotor activity studies with trichloroethylene (TCE).* A number of papers have measured locomotor activity and used functional observational batteries (FOBs) in order to obtain a more fine grained analysis of the motor behaviors that are impaired by TCE exposure. While exposure to TCE has been shown repeatedly to yield impairments in neuromuscular function acutely, there is very little evidence that the effects persist beyond termination of exposure.

One of the most extensive evaluations of TCE on innate neurobehavior was conducted by Moser et al. (1995, 2003) using FOB testing procedures. Moser et al. (1995) evaluated the effects of acute and subacute (14-day) oral gavage administration of TCE in adult female Fischer 344 rats. Testing was performed both 4 hours post TCE administration and 24 hours after TCE exposure, and a comparison of these two time points along with comparison between the first day and the last day of exposure provides insight into the persistence of effects observed. Various outcome measures were grouped into five domains: autonomic, activity, excitability, neuromuscular, and sensorimotor. Examples of tests included in each of these groupings are as follows: Autonomic—lacrimation, salivation, palpebral closure, pupil response, urination, and defecation; Activity—rearing, motor activity counts home cage position. Excitability—ease of removal, handling reactivity, arousal, clonic, and tonic movements; and Neuromuscular—gait score, righting reflex, fore and hindlimb grip strength, and landing foot splay. Sensorimotor-tail-pinch response, click response, touch response, and approach response. Scoring was performed on a 4-point scale ranging from "1" (normal) to "4" (rare occurrence for control subjects). In the acute exposure, the exposure doses utilized were 150, 500, 1,500, and 5,000 mg/kg TCE in corn oil. These doses represent 3, 10, 30, and 56% of the limit dose. For the 14-day subacute exposure, the doses used were 50, 150, 500, and 1,500 mg/kg. Such doses represent 1, 3, 10, and 30% of the limit dose for TCE.

The main finding for acute TCE administration is that a significant reduction in activity level occurred after the highest dose of TCE (5,000 mg/kg) only. This effect showed substantial recovery 24 hours after exposure though residual decrements in activity were noted.

Neuromuscular function as reflected in the gait score was also severely affected only at 5,000-mg/kg dose and only at the 4-hour test period. Sensorimotor function reflected in response to a sudden click, was abnormal at both 1,500 and 5,000 mg/kg with a slight difference observed at 1,500 mg/kg and a robust difference apparent at 5,000 mg/kg. Additional effects noted, but not shown quantitatively were abnormal home-cage posture, increased landing foot splay, impaired righting and decreased fore and hind limb grip strength. It is uncertain at which doses such effects were observed.

With the exception of sensorimotor function, these same categories were also disrupted in the subacute TCE administration portion of the study. The lack of effect of TCE on sensorimotor function with repeated TCE dosing might reflect either habituation, tolerance, or an unreliable measurement at one of the time points. Given the absence of effect at a range of exposure doses, a true dose-response relationship cannot be developed from these data.

In the subacute study, there are no clearly reliable dose-related differences observed between treated and control subjects. Rearing, a contributor to the activity domain, was elevated in the 500-mg/kg dose group, but was normal in the 1,500-mg/kg group. The neuromuscular domain was noted as significantly affected at 15 days, but it is not clear which subtest was abnormal. It appears that the limited group differences may be random among subjects unrelated to exposure condition.

In a follow-up study, Moser et al. (2003) treated female Fischer 344 rats with TCE by oral gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 mg/kg/d, and testing was undertaken either 4 hours following the first or 10th dose as well as 24 hours after these two time points. The authors identified several significant effects produced by TCE administration including a decrease in motor activity, tail pinch responsiveness, reactivity to handling, hind limb grip strength, and body weight. Rats administered TCE also showed significantly more piloerection, higher gait scores, lethality, body weight loss, and lacrimation compared to controls. Only effects observed 4 hours after the 10th exposure dose were presented by the authors, and no quantitative information of these measurements is provided.

Albee et al. (2006) exposed male and female Fischer 344 rats to 250-, 800-, and 2,500-ppm TCE for 6 hours/day, 5 days/week for 13 weeks. FOB was performed 4 days prior to exposure and then monthly. Auditory impairments found by others (e.g., Muijser et al., 2000; Rebert et al., 1995; Crofton et al., 1994; Crofton and Zhao, 1997; Fechter et al., 1998; Boyes et al., 2000) were replicated at the highest exposure dose, but treatment related differences in grip strength or landing foot splay were not demonstrated. The authors report slight increases in handling reactivity among female rats and slightly more activity than in controls at an intermediate time point, but apparently did not conduct systematic statistical analyses of these observations. In any event, there were no statistically significant effects on activity or reactivity by the end of exposure.

Kulig (1987) also failed to show significant effects of TCE inhalation exposure on markers of motor behavior. Wistar rats exposed to 500, 1,000, and 1,500 ppm for 16 hours/day, 5 days/week for 18 weeks failed to show changes in spontaneous activity, grip strength, or coordinated hind limb movement. Measurements were made every three weeks during the exposure period and occurred between 45 minutes and 180 minutes following the previous TCE inhalation exposure. This study establishes a NOAEL of 1,500-ppm TCE with an exposure duration of 16 hours/day.

D.2.6.2.2. ***Acute and subacute oral exposure to dichloroacetic acid on functional observational batteries (FOB).*** Moser et al. (1999) conducted a series of experiments on DCA ranging from acute to chronic exposures. The exposure doses used in the acute experiment were 100, 300, 1,000, and 2,000 mg/kg. In the repeated exposure studies (8 weeks−24 months), doses varied between 16 and 1,000 mg/kg/d. The authors showed pronounced neuromuscular changes in Long Evans and F344 rats dosed orally with the TCE metabolite, DCA, over a period ranging from 9 weeks to 24 months at different exposure doses. Using a multitude of exposure protocols which most commonly entailed daily exposures to DCA either by gavage or drinking water the authors identify effects that were "mostly limited" to the neuromuscular domain. These included disorders of gait, grip strength, foot splay and righting reflex that are dose and duration dependent. Data on gait abnormality and grip strength are presented in greatest detail. In adults exposed to DCA by gavage, gait scores were "somewhat abnormal" at the 7-week test in both the adult Long Evans rats receiving 300 and those receiving 1,000 mg/kg/d. There was no adverse effect in the rats receiving 100 mg/kg/d. In the chronic study, which entailed intake of DCA via drinking water yielding an estimated daily dose of 137 and 235 mg/kg/d "moderately to severely abnormal" gait was observed within 2 months of exposure and dosing was either reduced or discontinued because of the severity of toxicity. For the higher DCA dose, gait scores remained "severely abnormal" at the 24-month test time even though the DCA had been discontinued at the 6-month test time. Hindlimb grip strength was reduced to about ½ the control value in both exposure doses and remained reduced throughout the 24 months of testing even though DCA administration ceased at 6 months for the 235 mg/kg/d group. Forelimb grip strength showed a smaller and apparently reversible effect among DCA treated rats.

D.2.6.3. ***Locomotor Activity***

Wolff and Siegmund (1978) administered 182 mg/kg TCE (i.p.) in AB mice and observed a decrease in spontaneous locomotor activity. In this study, AB mice were injected with TCE 30 minutes prior to testing for spontaneous activity at one of 4 time points during a 24 hours/day (0600, 1200, 1800, and 2400 hours). Marked decreases (estimated 60−80% lower than control mice) in locomotor activity were reported in 15-minute test periods. The reduction in locomotion was particularly profound at all time intervals save for the onset of light (0600). Nevertheless, even at this early morning time point, activity was markedly reduced from control levels (60% lower than controls as approximated from a graph).

Moser et al. (1995, 2003) included locomotor activity as one of their measures of neurobehavioral effects of TCE given by gavage over a 10−14 day period. In the 1995 paper, female Fischer 344 rats were dosed either acutely with 150, 500, 1,500 or 5,000 mg/kg TCE or for 14 days with 50, 150, 500 or 1,500 mg/kg. In terms of the locomotor effects, they report that acute exposure produced impaired locomotor scores only at 5,000 mg/kg while in the subacute study, locomotion was impaired at the 500 mg/kg dose, but not at the 1,500 mg/kg dose. In the Moser (2003) study, it appears that 200 mg/kg TCE may actually have increased locomotor activity while the higher test doses (800 and 1,200 mg/kg) decreased activity in a dose related manner. What is common to both studies, however, is a depression in motor activity that occurs acutely following TCE administration and which may speak to the anesthetic if not central nervous system depressive effects of this solvent.

There are also a number of reports (Waseem et al., 2001; Fredriksson et al., 1993; Kulig, 1987) that failed to demonstrate impairment of motor activity or ability following TCE exposure. Waseem et al. (2001) failed to show effects of TCE given in the drinking water of Wistar rats over the course of a 90 day trial. While nominal solvent levels were 350, 700, and 1,400 ppm in the water, no estimate is provided of daily TCE intake or of the stability of the TCE solution over time. However, assuming a daily water intake of 25 mL/day and body weight of 330 g, these exposures would be estimated to be approximately 26, 52, and 105 mg/kg. These doses are far lower than those studied by Moser and colleagues.

Fredriksson et al. (1993) studied the effects of TCE given by oral gavage to male NMRI mice at doses of 50 and 290 mg/kg/d from postnatal Day 10−16 on locomotion assessed either on the day following exposure or at age 60 days. They found no significant effect of TCE on locomotor activity and no consistent effects on other motor behaviors (e.g., rearing).

Waseem et al. (2001) studied locomotor activity in Wistar rats exposed for up to 180 days to 376-ppm TCE by inhalation for 4 hours/day, 5 days/week and acutely intoxicated with TCE. Here the authors report seemingly inconsistent effects of TCE on locomotion. After 30 days of exposure, the treated rats show an increase in locomotor activity relative to control subjects. However, after 60 days of exposure they note a significant *increase* in distance traveled found among experimental subjects, but a decrease in horizontal activity in this experimental group. Moreover, the control subjects vary substantially in horizontal counts among the different time periods. No differences between the treatment groups are found after 180 days of exposure. It is difficult to understand the apparent discrepancy in results reported at 60 days of exposure.

D.2.7. Sleep and Mood Disorders

D.2.7.1. *Effects on Mood: Laboratory Animal Findings*

It is difficult to obtain comparable data of emotionality in laboratory studies. However, Moser et al. (2003) and Albee et al. (2006) both report increases in handling reactivity among rats exposed to TCE. In the Moser study, female Fischer 344 rats received TCE by oral gavage for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 mg/kg/d while Albee et al. (2006) exposed Fischer 344 rats to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm for 6 hours/day, 5 days/week for 13 weeks.

D.2.7.2. *Sleep Disturbances*

Arito et al. (1994) exposed male Wistar rats to 50-, 100-, and 300-ppm TCE for 8 hours/day, 5 days/week for 6 weeks and measured electroencephalographic (EEG) responses. EEG responses were used as a measure to determine the number of awake (wakefulness hours) and sleep hours. Exposure to all the TCE levels significantly decreased amount of time spent in wakefulness during the exposure period. Some carry over was observed in the 22-hour postexposure period with significant decreases in wakefulness seen at 100-ppm TCE. Significant changes in wakefulness-sleep elicited by the long-term exposure appeared at lower exposure levels. These data seem to identify a low dose of TCE that has anesthetic properties and established a LOAEL of 50 ppm for sleep changes.

D.2.8. Mechanistic Studies

D.2.8.1. *Dopaminergic (DA) Neurons*

In two separate animal studies, subchronic administration of TCE has resulted in a decrease of dopaminergic (DA) cells in both rats and mice. Although the mechanism for DA neurons resulting from TCE exposure is not elucidated, disruption of DA-containing neurons has been extensively studied with respect to Parkinson's Disease and parkinsonism. In addition to Parkinson's Disease, significant study of MPTP and of high-dose manganese toxicity provides strong evidence for extrapyramidal motor dysfunction accompanying loss of dopamine neurons in the substantia nigra. These databases may provide useful comparisons to the highly limited database with regard to TCE and dopamine neuron effects. The studies are presented in Table D-9.

Gash et al. (2007) assessed the effects of subchronic TCE administration on dopaminergic neurons in the central nervous system. Fischer 344 male rats were orally administered by gavage 1,000 mg/kg TCE in olive oil, 5 days/week for 6 weeks. Degenerative changes in DA containing neurons in the substantia nigra were reported as indexed by a 45% decrease in the number of tyrosine hydroxylase positive cells. Additionally, there was a decrease in the ratio of 3,4-dihydroxyphenylacetic acid, a metabolite of DA, to DA levels in the striatum. This shift in ratio, on the order of 35%, was significant by Student's t-test, suggesting a decrease in release and utilization of this neurotransmitter. While it is possible that long-term adaptation might occur with regard to release rates for DA, the loss of DA cells in the substantia nigra is viewed as a permanent toxic effect. The exposure level used in this study was limited to one high dose and more confidence in the outcome will depend upon replication and development of a dose-response relationship. If the results are replicated, they might be important in understanding mechanisms by which TCE produces neurotoxicity in the central nervous system. The functional significance of such cellular loss has not yet been determined through behavioral testing.

Guehl (1999) also reported persistent effects of TCE exposure on DA neurons. In this study, OF1 male mice (n = 10) were injected i.p. daily for 5 days/week for 4 weeks with TCE (400 mg/kg/d). Following a 7 day period when the subjects did not receive TCE, the mice were euthanized and tyrosine hydroxylase immunoreactivity was used to measure neuronal death in the substantia nigra pars compacta. Treated mice presented significant dopaminergic neuronal death (50%) in comparison with control mice based upon total cell counts conducted by an examiner blinded as to treatment group in six samples per subject. The statistical comparison appears to be by Student's t-test (only means, standard deviations, and a probability of $p < 0.001$ are reported). While this study appears to be consistent with that of Gash et al. (2007) there are some limitations of this study. Specifically, no photomicrographs are provided to assess adequacy of the histopathological material. Additionally, no dose-response data are available to characterize dose-response relationships or identify either a benchmark dose or NOAEL. Behavioral assessment aimed at determining functional significance was not determined.

The importance of these two studies suggesting death of dopaminergic neurons following TCE exposure may be addressable by human health studies because they suggest the potential for TCE to produce a parkinsonian syndrome.

D.2.8.2. *Gamma-Amino Butyric Acid (GABA) and Glutamatergic Neurons*

Disruption of GABAergic and glutamatergic neurons by toxicants can represent serious impairment as gamma-amino butyric acid (GABA) serves as a key inhibitory neurotransmitter while glutamate is equally important as an excitatory neurotoxicant. Moreover, elevations in glutamatergic release have been identified as an important process by which more general neurotoxicity can occur through a process identified as excitotoxicity. The data with regard to

TCE exposure and alteration in GABA and glutamate function is limited. The studies are presented in Table D-10.

Briving et al. (1986) conducted a chronic inhalation exposure in Mongolian gerbils to 50-and 150-ppm TCE continuously for 12 months and reported the changes in amino acids levels in the hippocampus and cerebellar vermis and on high affinity uptake of GABA and glutamate in those same structures. A dose related elevation of glutamine in the hippocampus of approximately 20% at 150 ppm was reported, but no other reliable changes in amino acids in either of these two structures. With regard to high affinity uptake of glutamate and GABA, there were no differences in the hippocampal uptake between control and treated gerbils although in the cerebellar vermis there was a dose related elevation in the high affinity uptake for both of these neurotransmitter. Glutamate uptake was increased about 50% at 50 ppm and 100% at 150 ppm. The corresponding increases for GABA were 69% and 74%. Since control tissue uptake is identified as being 100% rather than as an absolute rate, the ability to assess quality of the control data are limited. It is unclear if this finding in cerebellar vermis is also present in other brain tissues and should be studied further. If these findings are reliable, the changes in high affinity uptake in cerebellum for GABA and glutamate might represent alterations that could have functional outcomes. For example, alteration in GABA release and reuptake from the cerebellum might be consistent with acute alteration in vestibular function described below. However, there are presently no compelling data to support such a relationship.

The change in hippocampal glutamine levels is not readily interpretable. What is not clear from this paper is whether the alterations observed were acute effects observable only while subjects were intoxicated with TCE or whether they would persist once TCE had been removed from the neural tissue. This study used inhalation doses that were at least 1 order of magnitude lower than those required to produce auditory impairment.

A study by Shih et al. (2001) provides indirect evidence in male Mf1 mice that TCE exposure by injection might alter GABAergic function. The mice were injected i.p. with 250, 500, 1,000 and 2,000 mg/kg TCE in corn oil and the effect of these treatments on susceptibility to seizure induced by a variety of drugs was observed. Shih et al. report that doses of TCE as low as 250 mg/kg could reduce signs of seizure induced by picrotoxin, bicuculline, and pentylenetetrazol. These drugs are all GABAergic antagonists. TCE treatment had a more limited effect on seizure threshold induced by non-GABAergic convulsant drugs such as strychnine (glycine receptor antagonist), 4-aminopyridine (alcohol dehydrogenase inhibitor) and N-methyl-d-aspartate (glutamatergic agonist) than was observed with the GABAergic antagonists. While these data suggest the possibility that TCE could act at least acutely on GABAergic neurons, there are no direct measurements of such an effect. Moreover, there is no obvious relationship between these findings and those of Briving et al. (1986) with regard to increased high affinity uptake of glutamate and GABA in cerebellum. Beyond that fact, this study does not provide information regarding persistent effects of TCE on either seizure susceptibility or GABAergic function as all measurements were made acutely shortly following a single injection of TCE.

D.2.8.3. *Demyelination Following Trichloroethylene (TCE) Exposure*

Because of its anesthetic properties and lipophilicity, it is hypothesized that TCE may disrupt the lipid-rich sheaths that cover many central and peripheral nerves. This issue has also been studied both in specific cranial nerves known to be targets of TCE neurotoxicity (namely the trigeminal nerve) and in the central nervous system including the cerebral cortex, hippocampus and cerebellum in particular. For peripheral and cranial nerves, there are limited nerve conduction velocity studies that are relevant as a functional measure. For central pathways, the most common outcomes studied include histological endpoints and lipid profiles.

A significant difficulty in assessing these studies concerns the permanence or persistence of effect. There is a very large literature unrelated to TCE, which demonstrates the potential for repair of the myelin sheath and at least partial if not full recovery of function. In the studies where nerve myelin markers are assessed, it is not possible to determine if the effects are transient or persistent.

There are two published manuscripts (Isaacson and Taylor, 1989; Isaacson et al., 1990) that document selective hippocampal histopathology when Sprague Dawley rats are exposed to TCE within a developmental model. Both of these studies employed oral TCE administration via the drinking water. In Isaacson and Taylor, (1989), a combined prenatal and neonatal exposure was used while Isaacson's et al. (1990) report focused on a neonatal exposure. In addition, Ohta et al. (2001) presented evidence of altered hippocampal function in an *in vitro* preparation following acute *in vivo* TCE intoxication. The latter most manuscript details a shift in long term potentiation elicited by tetanic shocks to hippocampal slices *in vitro*. In the two developmental studies the exposure doses are expressed in terms of the concentration of TCE placed in the drinking water and the total daily dose is then estimated based upon average water intake by the subjects. However, since the subjects' body weight is not provided, it is not possible to estimate dosage on a mg/kg body weight basis.

Isaacson and Taylor (1989) examined the development of the hippocampus in neonatal rats that were exposed *in utero* and in the preweaning period to TCE via their dam. TCE was added to the drinking water of the dam and daily maternal doses are estimated based upon water intake of the dam as being 4 and 8.1 mg/day. Based upon body weight norms for 70-day old female Sprague Dawley rats, which would predict body weights of about 250 g at that age, such a dose might approach 16−32 mg/kg/d initially during pregnancy. Even if these assumptions hold true, it is not possible to determine how much TCE was received by the pups although the authors do provide an estimate of fetal exposure expressed as μg/mL of TCE, trichloroethanol, and trichloroacetic acid. The authors reported a 40% decline in myelinated fibers in the CA1 region of the hippocampus of the weanling rats. There was no effect of TCE treatment on myelination in several other brain regions including the internal capsule, optic tract or fornix and this effect appears to be restricted to the CA1 region of the hippocampus at the tested exposures.

In a second manuscript by that group (Isaacson et al., 1990), weanling rats were exposed to TCE via their drinking water at doses of 5.5 mg/day for 4 weeks or 5.5 mg/day for 4 weeks, a 2 week period with no TCE and then a final 2 weeks of exposure to 8.5 mg/day TCE. Spatial learning was studied using the Morris water maze and hippocampal myelination was examined histologically starting 1 day postexposure. The authors report that the subjects receiving a total of 6 weeks exposure to TCE showed *better* performance in the Morris swim test ($p < 0.05$) than did controls while the 4 week exposed subjects performed at the same level as did controls. Despite this apparent improvement in performance, histological examination of the hippocampus demonstrated a dose dependent relationship with hippocampal myelin being significantly reduced in the TCE exposed groups while normal myelin patterns were found in the internal capsule, optic tract and fornix. The authors did not evaluate the signs of gross toxicity in treated animals such as growth rate, which might have influenced hippocampal development.

Ohta et al. (2001) administered 300 or 1,000 mg/kg TCE, i.p., to male ddY mice. Twenty-four hours after TCE administration, the mice were sacrificed and hippocampal sections were prepared from the excised brains and long term potentiation was measured in the slices. A dose related reduction in the population spike was observed following a tetanic stimulation relative to the size of the population spike elicited in the TCE mice prior to tetany. The spike amplitude was reduced 14% in the 300 mg/kg TCE group and 26% in the 1,000 mg/kg group. Precisely how such a shift in excitability of hippocampal CA1 neurons relates to altered hippocampal function is not certain, but it does demonstrate that injection with 300 mg/kg TCE can have lingering consequences on the hippocampus at least 24 hours following i.p. administration.

A critical area for future study is the potential that TCE might have to produce demyelination in the central nervous system. While it is realistic to imagine that an anesthetic and lipophilic agent such as TCE might interact with lipid membranes and produce alterations, for example, in membrane fluidity at least at anesthetic levels, the data collected by Kyrklund and colleagues suggest that low doses of TCE (50 and 150 ppm chronically for 12 months, 320 ppm for 90 days, 510 ppm 8 hours/day for 5 months) might alter fatty acid metabolism in Sprague Dawley rats and Mongolian gerbils. Because they have not included high doses in their studies and because the low doses produce only sporadic significant effects and these tend to be of very small magnitude (5−10%) it is not certain that they are truly observing events with biological significance or whether they are observing random effects. A key problem in determining whether the effects under study are spurious or are due to ongoing exposure is that the magnitude and direction of the effect does not grow larger as exposure continues. It could be hypothesized that the alterations in fatty acid metabolism could be an underlying mechanism for demyelination. However, there is not enough evidence to determine if the changes in the lipid profiles lead to demyelination or if the observed effects are purely due to chance. Similarly, the size of statistically significant effects (5−12%) is generally modest. A broad dose-response analysis or the addition of a positive control group that is treated with an agent well-known to produce central demyelination would be important in order to characterize the potency of TCE as an agent that disrupts central nervous system lipid profiles.

Kyrklund and colleagues (e.g., 1986) have generally evaluated the hippocampus, cerebral cortex, cerebellum, and in some instances brainstem in adult gerbil. It is not apparent that one brain region is more vulnerable to the effects of TCE than is another region. While this group does not report significant changes in levels of cholesterol, neutral and acidic phospholipids or total lipid phospholipids, they do suggest a shift in lipid profiles between treated and untreated subjects. Similarly, inhalation exposure to trichloroethane at 1,200 ppm for 30 days (Kyrklund and Haglid, 1991) leads to sporadic changes in fatty acid profiles in Sprague Dawley rats. However, these changes are small and are not always in the same direction as the changes observed following trichloroethylene exposure. In the case of trichloroethane, a NOAEL of 320 ppm for 30 days 24 hours/day was observed and no other doses were evaluated (Kyrklund et al., 1988).

D.2.9. Summary Tables

Tables D-4 through D-8 summarize the animal studies by neurological domains (Table D-4—trigeminal nerve; Table D-5—ototoxicity; Table D-6—vestibular and visual systems; Table D-7—cognition; and Table D-8—psychomotor function and locomotor activity). For each table, the reference, exposure route, species, dose level, effects and NOAEL/LOAEL are provided. Tables D-9 through D-11 summarize mechanistic (Tables D-9 and D-11) and neurochemical studies (Table D-10). Brief summaries of developmental neurotoxicity studies are provided in Table D-12.

Table D-4. Summary of mammalian *in vivo* trigeminal nerve studies

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL: LOAEL	Effects
Barret et al., 1991	Direct Gastric Administration	Rat, Sprague-Dawley, female, 21	0, 2.5 g/kg, acute administration	LOAEL: 2.5 g/kg	Morphometric analysis was used for analyzing the trigeminal nerve. Increase in external and internal fiber diameter as well as myelin thickness was observed in the trigeminal nerve after TCE treatment.
Barret et al., 1992	Direct Gastric Administration	Rat, Sprague-Dawley, female, 18	0, 2.5 g/kg; 1 dose/d, 5 d/wk, 10 wks	LOAEL: 2.5 g/kg	Trigeminal nerve analyzed using morphometric analysis. Increased internode length and fiber diameter in class A fibers of the trigeminal nerve observed with TCE treatment. Changes in fatty acid composition also noted.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 2,500 ppm	No effect on trigeminal nerve function was noted at any exposure level.

Table D-5. Summary of mammalian *in vivo* ototoxicity studies

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Rebert et al., 1991	Inhalation	Rat, Long Evans, male, 10/group	Long Evans: 0, 1,600, 3,200 ppm; 12 h/d, 12 wk	Long Evans: NOAEL: 1,600 ppm; LOAEL: 3,200 ppm	BAERs were measured. Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1).
		Rat, F344, male, 4−5/group	F344: 0, 2000, 3200 ppm; 12 h/d, 3 wk	F344: LOAEL: 2,000 ppm	
Rebert et al., 1993		Rat, Long Evans, male, 9/group	0, 2,500, 3,000, 3,500 ppm; 8 h/d, 5 d	NOAEL: 2,500 ppm LOAEL: 3,000 ppm	BAERs were measured 1−2 wk postexposure to assess auditory function. Significant decreases in BAERs were noted with TCE exposure.
Rebert et al., 1995		Rat, Long Evans, male, 9/group	0, 2,800 ppm; 8 h/d, 5 d	LOAEL: 2,800 ppm	BAER measured 2−14 d postexposure at a 16-kHz tone. Hearing loss ranged from 55−85 dB.
Crofton et al., 1994		Rat, Long Evans, male, 7−8/group	0, 3,500 ppm TCE; 8 h/d, 5 d	LOAEL: 3,500 ppm	BAER measured and auditory thresholds determined 5−8 wk postexposure. Selective impairment of auditory function for mid-frequency tones (8 and 16 kHz).
Crofton and Zhao, 1997; Boyes et al., 2000	Inhalation	Rat, Long Evans, male, 9−12/group	0, 4,000, 6,000, 8,000 ppm; 6 h	NOAEL: 6,000 ppm LOAEL: 8,000 ppm	Auditory thresholds as measured by BAERs for the 16-kHz tone increased with TCE exposure.
		Rat, Long Evans, male, 8−10/group	0, 1,600, 2,400, 3,200 ppm; 6 h/d, 5 d	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long Evans, male, 8−10/group	0, 800, 1,600, 2,400, 3,200 ppm; 6 h/d, 5 d/wk, 4 wk	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long Evans, male, 8−10/group	0, 800, 1,600, 2,400, 3,200 ppm; 6 h/d, 5 d/wk, 13 wk	NOAEL: 1,600 ppm LOAEL: 2,400 ppm	

Table D-5. Summary of mammalian *in vivo* ototoxicity studies (continued)

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL*	Effects
Fechter et al., 1998	Inhalation	Rat, Long Evans, male, 12/group	0, 4,000 ppm; 6 h/d, 5 d	LOAEL: 4,000 ppm	Cochlear function measured 5−7 wk after exposure. Loss of spiral ganglion cells noted. Auditory function was significantly decreased as measured by compound action potentials.
Jaspers et al., 1993	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 12/group	0, 1,500, 3,000 ppm; 18 h/d, 5 d/wk, 3 wk	LOAEL: 1,500 ppm	Auditory function assessed repeatedly 1−5 wk postexposure for 5-, 20-, and 35-kHz tones; No effect at 5 or 35 kHz; Decreased auditory sensitivity at 20 kHz.
Muijser et al., 2000	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 8	0, 3,000 ppm	LOAEL: 3,000 ppm	Auditory sensitivity decreased with TCE exposure at 4-, 8-, 16-, and 20-kHz tones.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 800 ppm LOAEL: 2,500 ppm	Mild frequency specific hearing deficits; Focal loss of hair cells and cochlear lesions.
Yamamura et al., 1983	Inhalation	Guinea Pig, albino Hartley, male, 7−10/group	0, 6,000, 12,000, 17,000 ppm; 4 h/d, 5 d	NOAEL: 17,000 ppm	No change in auditory sensitivity at any exposure level as measured by cochlear action potentials and microphonics.

Table D-6. Summary of mammalian sensory studies—vestibular and visual systems

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Vestibular system studies					
Tham et al., 1979	Intravenous	Rabbit, strain unknown, sex unspecified, 19	1−5 mg/kg/min	---	Positional nystagmus developed once blood levels reached 30 ppm.
Tham et al., 1984	Intravenous	Rat, Sprague-Dawley, female, 11	80 μg/kg/min	---	Excitatory effects on the vestibule-oculomotor reflex. Threshold effect at blood (TCE) of 120 ppm or 0.9 mM/L.
Niklasson et al., 1993	Inhalation	Rat, strain unknown, male and female, 28	0, 2,700, 4,200, 6,000, 7,200 ppm; 1 h	LOAEL: 2,700 ppm	Increased ability to produce nystagmus.
Umezu et al., 1997	Intraperitoneal	Mouse, ICR, male, 116	0, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 250 mg/kg LOAEL: 500 mg/kg	Decreased equilibrium and coordination as measured by the Bridge test (staying time on an elevated balance beam).
Visual system studies					
Rebert et al., 1991	Inhalation	Rat, Long Evans, male, 10/group	0, 1,600, 3,200 ppm; 12 h/d, 12 weeks	NOAEL: 3,200 ppm	No effect on visual function as measured by visual evoked potential changes.
		Rat, F344, male, 4−5/group	0, 2,000, 3,200 ppm; 12 h/d, 3 wk	NOAEL: 3,200 ppm	
Boyes et al., 2003	Inhalation	Rat, Long Evans, male, 9−10/group	0 ppm, 4 h; 1,000 ppm, 4 h; 2,000 ppm, 2 h; 3,000 ppm, 1.3 h; 4,000 ppm, 1 h	LOAEL: 1,000 ppm, 4 h	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials.
Boyes et al., 2005	Inhalation	Rat, Long Evans, male, 8−10/group	0 ppm, 4 h; 500 ppm, 4 h; 1,000 ppm, 4 h; 2,000 ppm, 2 h; 3,000 ppm, 1.3 h; 4,000 ppm, 1 h; 5,000 ppm, 0.8 h	LOAEL: 500 ppm, 4 h	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials.
Blain et al., 1994	Inhalation	Rabbit, New Zealand albino, male, 6−8/group	0, 350, 700 ppm; 4 h/d, 4 d/wk, 12 wk	LOAEL: 350 ppm	Significant effects noted in visual function as measured by ERG and OPs immediately after exposure. No differences in ERG or OP measurements were noted at 6 wk post-TCE exposure.

Table D-7. Summary of mammalian cognition studies

Reference	Exposure route	Species, strain, sex, number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Kjellstrand et al., 1980	Inhalation	Gerbil, Mongolian, males and females, 12/sex/dose	0, 320 ppm; 9 mos, continuous (24 h/d) except 1−2 h/wk for cage cleaning	NOAEL: 320 ppm	No significant effect on spatial memory (radial arm maze).
Kulig et al., 1987	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, 1,500 ppm; 16 h/d, 5 d/wk, 18 wk	NOAEL: 500 ppm LOAEL: 1,000 ppm	Increased latency time in the two-choice visual discrimination task (cognitive disruption and/or motor activity related effect).
Isaacson et al., 1990	Oral, drinking water	Rat, Sprague Dawley, male, 12/dose	(1) 0 mg/kg/d, 8 wk (2) 5.5 mg/d (47 mg/kg/d*), 4 wk + 0 mg/kg/d, 4 wk (3) 5.5 mg/d, 4 wk (47 mg/kg/d*) + 0 mg/kg/d, 2 wk + 8.5 mg/d (24 mg/kg/d),* 2 wk	NOAEL: 5.5 mg/d, 4 wk spatial learning LOAEL: 5.5 mg/d hippocampal demyelination	Decreased latency to find platform in the Morris water maze (Group #3); Hippocampal demyelination observed in all TCE-treated groups.
Kishi et al., 1993	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, 4,000 ppm, 4 h	LOAEL: 250 ppm	Decreased lever presses and avoidance responses in a shock avoidance task.
Umezu et al., 1997	Intraperitoneal	Mouse, ICR, male, 6 exposed to all treatments	0, 125, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg	Decreased response rate in an operant response-cognitive task.
Ohta et al., 2001	Intraperitoneal	Mouse, ddY, male, 5/group	0, 300, 1,000 mg/kg, sacrificed 24 h after injection	LOAEL: 300 mg/kg	Decreased response (LTP response) to tetanic stimulation in the hippocampus.
Oshiro et al., 2004	Inhalation	Rat, Long Evans, male, 24	0, 1,600, 2,400 ppm; 6 h/d, 5 d/wk, 4 wk	NOAEL: 2,400 ppm	No change in reaction time in signal detection task and when challenged with amphetamine, no change in response from control.

*mg/kg/d conversion estimated from average male Sprague-Dawley rat body weight from ages 21−49 days (118 g) for the 5.5 mg dosing period and ages 63−78 days (354 g) for the 8.5 mg dosing period.

Table D-8. Summary of mammalian psychomotor function, locomotor activity, and reaction time studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Savolainen et al., 1977	Inhalation	Rat, Sprague Dawley, male, 10	0, 200 ppm; 6 h/d, 4 d	LOAEL: 200 ppm	Increased frequency of preening, rearing, and ambulation. Increased preening time.
Wolff and Siegmund, 1978	Intraperitoneal	Mouse, AB, male, 144	0, 182 mg/kg, tested 30 min after injection	LOAEL: 182 mg/kg	Decreased spontaneous motor activity.
Kulig et al., 1987	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, 1,500 ppm; 16 h/d, 5 d/wk, 18 wk	NOAEL: 1,500 ppm	No change in spontaneous activity, grip strength or hindlimb movement.
Motohashi and Miyazaki, 1990	Intraperitoneal	Rat, Wistar, male, 44	0, 1.2 g/kg, tested 30 min after injection	LOAEL: 1.2 g/kg	Increased incidence of rats slipping in the inclined plane test.
			0, 1.2 g/kg/d, 3 d	LOAEL: 1.2 g/kg	Decreased spontaneous motor activity.
Fredericksson et al., 1993	Oral	Mouse, NMRI, male, 12 (3−4 litters)	0, 50, 290 mg/kg/d, at Days 10−16	---	Decreased rearing; No evidence of dose response.
Moser et al., 1995	Oral	Rat, Fischer 344, female, 8/dose	0, 150, 500, 1,500, 5,000 mg/kg, 1 dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity; Neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, 1,500 mg/kg/d, 14 d	NOAEL: 150 mg/kg/d LOAEL: 500 mg/kg/d	Increased rearing activity.
Bushnell, 1997	Inhalation	Rat, Long Evans, male, 12	0, 400, 800, 1,200, 1,600, 2,000, 2,400 ppm, 1-h/test day, 4 consecutive test days, 2 wk	NOAEL: 800 ppm LOAEL: 1,200 ppm	Decreased sensitivity and increased response time in the signal detection task.

Table D-8. Summary of mammalian psychomotor function, locomotor activity, and reaction time studies (continued)

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL*	Effects
Umezu et al., 1997	Intraperitoneal	Mouse, ICR, male, 6 exposed to all treatments	0, 2,000, 4,000, 5,000 mg/kg – loss of righting reflex measure	LOAEL: 2,000 mg/kg – loss of righting reflex	Loss of righting reflex, decreased operant responses, increased punished responding.
			0, 62.5, 125, 250, 500, 1,000 mg/kg, single dose and evaluated 30 min postadministrati on	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg – operant behavior NOAEL: 125 mg/kg LOAEL: 250 mg/kg – punished responding	
Bushnell and Oshiro, 2000	Inhalation	Rat, Long Evans, male, 32	0, 2,000, 2,400 ppm; 70 min/d, 9 d	LOAEL: 2,000 ppm	Decreased performance on the signal detection task. Increased response time and decreased response rate.
Nunes et al., 2001	Oral	Rat, Sprague Dawley, male, 10/group	0, 2,000 mg/kg/d, 7 d	LOAEL: 2,000 mg/kg/d	Increased foot splay. No change in any other FOB parameter (e.g., piloerection, activity, reactivity to handling).
Waseem et al., 2001	Oral	Rat, Wistar, male, 8/group	0, 350, 700, 1,400 ppm in drinking water for 90 d	NOAEL: 1,400 ppm	No significant effect on spontaneous locomotor activity.
	Inhalation	Rat, Wistar, male, 6/group	0, 376 ppm for up to 180 d	LOAEL: 376 ppm	Changes in locomotor activity but not consistent when measured over the 180-day period.
Moser et al., 2003	Oral	Rat, Fischer 344, female, 10/group	0, 40, 200, 800, 1,200 mg/kg/d, 10 d	---	Decreased motor activity; Decreased sensitivity; Increased abnormality in gait; Adverse changes in several FOB parameters.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm	NOAEL: 2,500 ppm	No change in any FOB measured parameter.

Table D-9. Summary of mammalian *in vivo* dopamine neuronal studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Guehl et al., 1999	Intraperitoneal administration	Mouse, OF1, male, 10	0, 400 mg/kg	LOAEL: 400 mg/kg	Significant dopaminergic neuronal death in substantia nigra.
Gash et al., 2007	Oral	Rat, Fischer 344, male, 17/group	0, 1,000 mg/kg	LOAEL: 1,000 mg/kg	Degeneration of dopamine-containing neurons in substantia nigra.

Table D-10. Summary of neurochemical effects with TCE exposure

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
In vivo studies					
Shih et al., 2001	Intraperitoneal	Mouse, Mf1, male, 6/group	0, 250 500, 1,000, 2,000 mg/kg, 15 min; followed by tail infusion of PTZ (5 mg/mL), picrotoxin (0.8 mg/mL), bicuculline (0.06 mg/mL), strychnine (0.05 mg/mL), 4-AP (2 mg/mL), or NMDA (8 mg/mL)	---	Increased threshold for seizure appearance with TCE pretreatment for all convulsants. Effects strongest on the $GABA_A$ antagonists, PTZ, picrotoxin, and bicuculline suggesting $GABA_A$ receptor involvement. NMDA and glycine Rc involvement also suggested.
Briving et al., 1986	Inhalation	Gerbils, Mongolian, male and female, 6/group	0, 50, 150 ppm, continuous, 24 h/d, 12 mos	NOAEL: 50 ppm; LOAEL: 150 ppm for glutamate levels in hippocampus NOAEL: 150 ppm for glutamate and GABA uptake in hippocampus LOAEL: 50 ppm for glutamate and GABA uptake in cerebellar vermis	Increased glutamate levels in the hippocampus. Increased glutamate and GABA uptake in the cerebellar vermis.
Subramoniam et al., 1989	Oral	Rat, Wistar, female,	0, 1,000 mg/kg, 2 or 20 h 0, 1,000 mg/kg/d, 5 d/wk, 1 y	---	PI and PIP2 decreased by 24 and 17% at 2 h. PI and PIP2 increased by 22 and 38% at 20 h. PI, PIP, and PIP2 reduced by 52,23, and 45% in 1-yr study.
Kjellstrand et al., 1987	Inhalation	Mouse, NMRI, male	0, 150, 300 ppm, 24 h/d, 4 or 24 d	LOAEL: 150 ppm, 4 and 24 d	Sciatic nerve regeneration was inhibited in both mice and rats.
		Rat, Sprague-Dawley, female	0, 300 ppm, 24 h/d, 4 or 24 d	NOAEL: 300 ppm, 4 d LOAEL: 300 ppm, 24 d	

Table D-10. Summary of neurochemical effects with TCE exposure (continued)

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL*	Effects
Haglid et al., 1981	Inhalation	Gerbil, Mogolian, male and female, 6−7/group	0, 60, 320 ppm, 24 h/d, 7 d/wk, 3 mos	LOAEL: 60 ppm, brain protein changes NOAEL: 60 ppm; LOAEL: 320 ppm, brain DNA changes	(1) Decreases in total brain soluble protein whereas increase in S100 protein. (2) Elevated DNA in cerebellar vermis and sensory motor cortex.

Table D-11. Summary of *in vitro* ion channel effects with TCE exposure

Reference	Cellular system	Neuronal channel/ receptor	Concentrations	Effects
In vitro studies				
Shafer et al., 2005	PC12 cells	Voltage sensitive calcium channels (VSCC)	0, 500, 1,000, 1,500, 2,000 μM	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of -70 mV.
Beckstead et al., 2000	*Xenopus* oocytes	Human recombinant Glycine receptor α1, $GABA_A$ receptors, α1β1, α1β2γ2L	0, 390 μM	50% potentiation of the $GABA_A$ receptors; 100% potentiation of the glycine receptor.
Lopreato et al., 2003	*Xenopus* oocytes	Human recombinant serotonin 3A receptor	???	Potentiation of serotonin receptor function.
Krasowski and Harrison, 2000	Human embryonic kidney 293 cells	Human recombinant Glycine receptor α1, $GABA_A$ receptors α2β1	Not provided	Potentiation of glycine receptor function with an EC_{50} of 0.65 ± 0.05 mM. Potentiation of $GABA_A$ receptor function with an EC_{50} of 0.85 ± 0.2.

EC_{50} = median effective concentration.

Table D-12. Summary of mammalian *in vivo* developmental neurotoxicity studies—oral exposures

Reference	Species/strain/ sex/number	Dose level/ exposure duration	Route/vehicle	NOAEL; LOAEL[a]	Effects
Fredriksson et al., 1993	Mouse, NMRI, male pups, 12 pups from 3−4 different litters/group	0, 50, or 290 mg/kg-d PND 10−16	Gavage in a 20% fat emulsion prepared from egg lecithin and peanut oil	Dev. LOAEL: 50 mg/kg/d	Rearing activity sig. ↓ at both dose levels on PND 60.
George et al., 1986	Rat, F334, male and female, 20 pairs/treatment group, 40 controls/sex	0, 0.15, 0.30, or 0.60% microencapsulated TCE. Breeders exposed 1 wk premating, then for 13 wk; pregnant ♀s throughout pregnancy (i.e., 18-wk total).	Dietary	LOAEL: 0.15%	Open field testing in pups: a sig. dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test device.
Isaacson and Taylor, 1989	Rat, Sprague-Dawley, females, 6 dams/group	0, 312, or 625 mg/L. (0, 4.0, or 8.1 mg/d)[b] Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOAEL: 312 mg/L	Sig. ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.
Noland-Gerbec et al., 1986	Rat, Sprague-Dawley, females, 9−11 dams/group	0, 312 mg/L (Avg. total intake of dams: 825 mg TCE over 61 d.)[b] Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOEL: 312 mg/L	Sig. ↓ uptake of ^{3}H-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.
Taylor et al., 1985	Rat, Sprague-Dawley, females, no. dams/group not reported	0, 312, 625, and 1,250 mg/L Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOAEL: 312 mg/L	Exploratory behavior sig. ↑ in 60- and 90-d old male rats at all treatment levels. Locomotor activity was higher in rats from dams exposed to 1,250-ppm TCE.

[a]NOAEL, LOAEL, and LOEL (lowest-observed-effect level) are based upon reported study findings.
[b]Dose conversions provided by study author(s).

PND = postnatal day.

D.3. REFERENCES

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APPENDIX E

Analysis of Liver and Coexposure Issues for the TCE Toxicological Review

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FOREWORD

The purpose of this Appendix is to provide scientific support and rationale for the hazard and dose-response sections of the Toxicological Review of Trichloroethylene (TCE) regarding liver effects and those of coexposures. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of TCE. Please refer to the Toxicological Review of TCE for characterization of EPA's overall confidence in the quantitative and qualitative aspects of hazard and dose-response for TCE-induced liver effects. Matters considered in this appendix include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the scientific issues regarding the data and MOA considerations for experimental animal data for liver effects in the TCE assessment.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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APPENDIX E. ANALYSIS OF LIVER AND COEXPOSURE ISSUES FOR THE TCE TOXICOLOGICAL REVIEW

E.1. BASIC PHYSIOLOGY AND FUNCTION OF THE LIVER—A STORY OF HETEROGENEITY

The liver is a complex organ whose normal function and heterogeneity are key to understanding and putting into context perturbations by trichloroethylene (TCE), cancer biology, and variations in response observed and anticipated for susceptible life stages and background conditions.

E.1.1. Heterogeneity of Hepatocytes and Zonal Differences in Function and Ploidy

Malarkey et al. (2005) state that (1) the liver transcriptome (i.e., genes expressed as measured by mRNA) is believed only second to the brain in its complexity and includes about 25−40% of the approximately 50,000 mammalian genes, (2) during disease states the transcriptome can double or triple and its increased complexity is due not only to differential gene expression (up- and down-regulation of genes) but also to the mRNA contributions from the heterogeneous cell populations in the liver, and (3) when one considers that over a dozen cell types comprise the liver in varying proportions, particularly in disease states, knowledge about the cell types and cell-specific gene expression profiles help unravel the complex genomic and protenomic data sets. Gradients of gene and protein activity varying from the periportal region to the centrilobular region also exist for sinusoidal endothelial cells, Kuffper cells, hepatic stellate cells, and the matrix in the space of Disse. Malarkey et al. (2005) also estimate that hepatocytes constitute 60%, sinusoidal endothelial cells 20%, Kupffer cells 15%, and stellate cells 5% of liver cells. Therefore, in experimental paradigms where liver homogenates are used for the determination of “changes in liver,” gene expression, or other parameters the individual changes from cells residing in differing zones and by differing cell type is lost. Malarkey et al. (2005) define the need to better characterize the histological cellular components of the tissues from which mRNA and protein is extracted and referred to “phenotypic anchoring” and cite acetaminophen as a “model hepatotoxicant under study to assess the strengths and weaknesses of genomics and proteinomics technologies” as well as “a good example for understanding and utilizing phenotypic anchoring to better understand genomics data.” After acetaminophen exposure “there is an unexplained and striking inter and intralobular variability in acute hepatic necrosis with some regions having massive necrosis and adjacent areas within the same lobe or other lobes showing no injury at all.” Malarkey et al. (2005) go on to cite similar lobular variability in response for “copper distribution, iron and phosphorous, chemical and spontaneous carcinogenesis, cirrhosis and regeneration" and suggest that although uncertain "factors such as portal streamlining of blood to the liver, redistribution of blood to core of the liver secondary to nerve stimulation, and exposures during fetal development and possibly lobular gradients are important." Hepatic interlobe differences exist for initiating agents in terms of DNA alkylation and cell replication. In the rat, diethylnitrosamine (DEN) alkylation has been reported to occur preferentially in the left and right median lobes, while cell replication was higher in the right median and right anterior lobes (Richardson et al., 1986). Richardson et al. (1986) reported that exposure to DEN induced a 100% incidence of hepatocellular carcinoma (HCC) in the left, caudate, left median and right median lobes of the liver by 20 weeks versus only 30% in the right anterior and right posterior hepatic lobes. There was a reported interlobe difference in adduct formation, cell proliferation, liver lobe weight gain, number and size of γ-glutamyltranspeptidase (GGT)+ foci, and carbon 14 labeling from a single dose of DEN. Richardson et al. (1986) suggest that many growth-selection studies utilizing the liver to evaluate the carcinogenic potential of a chemical often focus on only one or two of the hepatic lobes, which is especially true for partial hepatectomy, and that for DEN and possibly other chemicals this procedure removes the lobes most likely to get tumors. Thus, the "distribution of toxic insult may not be correctly assessed with random sampling of the liver tissue for microarray gene expression analysis" (Malarkey et al., 2005) and certainly any such distributional differences are lost in studies of whole-liver homogenates.

The liver is normally quiescent with few hepatocytes undergoing mitosis and, as described below, normally occurring in the periportal areas of the liver. Mitosis is observed only in approximately one in every 20,000 hepatocytes in adult liver (Columbano and Ledda-Columbano, 2003). The studies of Schwartz-Arad et al. (1989), Zajicek et al. (1991), Zajicek and Schwartz-Arad (1990), and Zajicek et al. (1989) have specifically examined the birth, death, and relationship to zone of hepatocytes as the "hepatic streaming theory." They report that hepatocytes and littoral cells continuously steam from the portal tract toward the terminal hepatic vein and that the hepatocyte differentiates as it goes with biological age closely related to cell differentiation. In other words, the acinus may be represented by a tube with two orifices: for cell inflow situated at the portal tract rim and other for cell outflow, at the terminal hepatic vein with hepatocytes streaming through the tube in an orderly fashion. In normal liver, cell proliferation is suggested as the only driving force of this flow with each mitosis associated with displacement of the cells by one cell location and the greater the cell production, the faster the flow and visa versa (Zajicek et al., 1991). Thus, the microscopic section of the liver "displays an instantaneous image of a tissue in flux" (Schwartz-Arad et al., 1989). Schwartz-Arad et al. (1989) further suggest that

> throughout its life the hepatocyte traverses three acinus zones; in each it is engaged in different metabolic activity. When young it performs among other functions gluconeogenesis, which is found in zone 1 hepatocytes (i.e. periportal), and when old it turns into a zone 3 cell (i.e., pericentral), with a pronounced glycolitic make up. The three zones thus represent differentiation stages of the hepatocyte, and since they differ by their distance from the origin, e.g. zone 2 (i.e., midzonal) is more distant than zone 1, again, hepatocyte differentiation is proportional to its distance.

Chen et al. (1995) report that

> Hepatocytes are a heterogeneous population that are composed of cells expressing different patterns of genes. For example, gamma-glutamyl transpeptidase and genes related to gluconeogenesis are expressed preferential in periportal hepatocytes, whereas enzymes related to glycolysis are more abundant in the centrilobular area. Glutamine synthetase is expressed in a small number of hepatocytes surrounding the central veins. Most cytochrome p450 enzymes are expressed or induced preferentially in centrilobular hepatocytes relative to periportal hepatocytes.

Along with changes in metabolic function, Vielhauer et al. (2001) reported that there is evidence of zonal differences in carcinogen DNA effects and, also, chemical-specific differences for DNA repair enzyme and that enhanced DNA repair is a general feature of many carcinogenic states including the enzymes that repair alkylating agents but also oxidative repair. As part of this process of differentiation and as livers age, the hepatocyte changes and increases its ploidy with polyploid cells predominant in zone 2 of the acinus (Schwartz-Arad et al., 1989). The reported decrease in DNA absorbance in zone 3 may be due to (1) a decline in chromatin affinity to the dye, (2) cell death, and (3) DNA exit from intact cells and Zajicek and Schwartz-Arad (1990) suggest that the fewer metabolic demands in Zone 3, under normal conditions, causes the cell to "deamplify" its genes and for DNA excess to leak out cells adjacent to the terminal hepatic vein or to be eliminated by apoptosis reflecting cell death. Thus, the three acinus zones represent differentiation states of one and the same hepatocyte, which increase ploidy as functional demands change. Zajicek and Schwartz-Arad (1990) also report that nuclear size is generally proportional to DNA content and that as DNA accumulates, the nucleus enlarges. This has import for histopathological descriptions of hepatocellular hypertrophy and attendant nuclear changes after toxic insult as well.

The gene amplification associated with polyploidy is manifested by DNA accumulation that involves the entire genome (Zajicek and Schwartz-Arad, 1990). Polyploidization is always attended by the intensification of the transcription and translation and in rat liver the amino acid label and activity of many enzymes increases proportionately to their ploidy. "Individual chromosomes of a tetraploid genome of a hepatocyte reduplicate in the same sequence as in a diploid one. In this case the properties of the chromosomes evidently remain unchanged and polyploidy only means doubling the indexes of the diploid genome" (Brodsky and Uryvaeva, 1977). Polyploidy will be manifested in the liver by either increases in the number of chromosomes per nucleus in an individual cell or by the appearance of two nuclei in a single cell. Most cell polyploidization occurs in youth with mitotic polyploidization occurring predominantly from 2 to 3 weeks postnatally and increases with age in mice (Brodsky and Uryvaeva, 1977). Hepatocytes progress through a modified or polyploidizing cell cycle which contains gaps and S-phases, but proceeds without cytokinesis. The result is the formation of the first polyploidy cell, which is binucleated with diploid nuclei and has increased cell ploidy but not cell number. The subsequent proliferation of bi-nucleated hepatocytes occurs with a fusion of mitotic nuclei during metaphase that gives rise to mononucleated cells with higher levels of ploidy. Thus, during normal liver ontogenesis, a polyploidizing cell cycle without cytokinesis alternates with a mitotic cycle of binucleated cells and results in progressive and irreversible increases in either cell or nuclear ploidy (Brodsky and Uryvaeva, 1977).

Polyploidization of the liver occurs during maturation in rodents and therefore, experimental paradigms that treat or examine rodent liver during that period should take into consideration the normally changing baseline of polyploidy in the liver. The development of polyploidy has been correlated in rodents to correspond with maturation. Brodsky and Uryvaeva (1977) report it is cells with diploid nuclei that proliferate in young mice, but that among the newly formed cells, the percentage of those with tetraploid nuclei is high. By 1 month, most mice (CBA/C57BL mice) already have a polyploid parenchyma, but binucleate cells with diploid nuclei predominate. In adult mice, the ploidy class with the highest percentage of hepatocytes was the 4n X 2 class. The intensive proliferation of diploid hepatocytes occurs only in baby mice during the first 2 weeks of life and then toward 1 month, the diploid cells cease to maintain themselves and transform into polyploid cells. In aged animals, the parenchyma retains only 0.02 percent of the diploid cells of the newborn animal. While the weight of the liver increases almost 30 times within 2 years, the number of cells increase much less than the weight or mean ploidy. Hence, the postnatal growth of the liver parenchyma is due to cell polyploidization (Brodsky and Uryvaeva, 1977). In male Wistar rats fetal hepatocytes (22 days gestation) were reported to be 85.3% diploid (2n) and 7.4% polyploid (4n + 8n) cells with 7.3% of cells in S-phase (S1 and S2). By one month of age (25-day old suckling rats) there were 92.9% diploid and 2.5% polyploid, at 2 months 47.5% diploid and 50.9% polyploid, at 6 months 29.1% diploid and 69.6% polyploid, and by 8 months 11.1% diploid and 87.3% polyploidy (Sanz et al., 1996). However, mouse and rat differ in their polyploidization.

> In the mouse, which has a higher degree of polyploidy than the rats, the scheme of polyploidization differs in that each cell class, including mononucleate cells, forms from the preceding one without being supplemented by self-maintenance. Each cell class is regarded as the cell clone and it is implied that the cells of each class have the same mitotic history and originate from diploid initiator cells with similar properties. In this model 1 reproduction would give a 2n × 2 cell, the second reproduction a 4n cell, and third reproduction a 4n X 2 cell all coming from an originator diploid cell (Brodsky and Uryvaeva, 1977).

The cell polyploidy is most extensive in mouse liver, but also common for rat and humans livers. The livers of young and aged mice differ considerably in the ploidy of the parenchymal cells, but still perform fundamentally the same functions. In some mammals, such as the mouse, rats, dog and human, the liver is formed of polyploid hepatocytes. In others, for example, guinea pig and cats, the same functions are performed by diploid cells (Brodsky and Uryvaeva, 1977). One obvious consequence of polyploidization is enlargement of the cells. The volume of the nucleus and cytoplasm usually increases proportionately to the increased in the number of chromosome sets with polyploidy reducing the surface/volume ratio. The labeling of tritium doubles with the doubling of the number of chromosomes in the hepatocyte nucleus (Brodsky and Uryvaeva, 1977). Kudryavtsev et al. (1993) have reported that the average levels of cell and nuclear ploidy are relatively lower in humans than in rodent but the pattern of hepatocyte polyploidization is similar and at maturity and especially during aging, the rate of hepatocyte polyploidization increases with elderly individuals having binucleated and polyploid hepatocytes constituting about one-half of liver parenchyma. Gramantieri et al. (1996) report that in adult human liver a certain degree of polyploidization is physiological; the polyploidy compartment (average 33% of the total hepatocytes) includes both mononucleated (28%) and binucleated (72%) cells and the average percentage of binucleated cells in the total hepatocyte population is 24% (Melchiorri et al., 1994). Historically, aging in human liver has been characterized by fewer and larger hepatocytes, increased nuclear polyploidy and a higher index of binucleate hepatocytes (Popper, 1986) but Schmucker (2005) notes that data concerning the effect of aging on hepatocyte volume in rodent and humans are in conflict with some showing increases volume to be unchanged and to increase by 25% by age 60 by others in humans. The irreversibility of hepatocyte polyploidy has been used in efforts to identify the origin of tumor progenitor cells (diploid vs. polyploidy) (see Section E.3.1.8, below). The associations with polyploidy and disease have been an active area of study in cancer mode-of-action (MOA) studies (see Sections E.3.1.4 and E.3.3.1, below).

Not only are polyploid cells most abundant in zone 2 of the liver acinus and increase in number with age, but polyploid cells have been reported to be more abundant following a number of toxic insults and exposure to chemical carcinogens. Wanson et al. (1980) reported that one of the earliest lesions obtained in the liver after *N*-nitrosomorpholine treatment development of hypertrophic parenchymal cells presenting a high degree of ploidy. Gupta (2000) reports hepatic polyploidy is often encountered in the presence of liver disease and that for animals and people, polyploidy is observed during advancement of liver injury due to cirrhosis or other chronic liver disease (often described as large-cell dysplasia referring to nuclear and cytoplasmic enlargement, nuclear pleomorphisms and multinucleation and probably representing increased prevalence of polyploidy cells) and in old animals with toxic liver injury and impaired recovery. Gorla et al. (2001) report that weaning and commencement of feeding, compensatory liver hypertrophy following partial hepatectomy, toxin and drug-induced liver disease, and administration of specific growth factors and hormones may induce hepatic polyploidy. They go on to state that “although liver growth control has long been studied, whether the replication potential of polyploidy hepatocytes is altered remains unresolved, in part, owing to difficulties in distinguishing between cellular DNA synthesis and generation of daughter cells.” Following CCL4 intoxication, the liver ploidy rises and more cells become binucleate (Zajicek et al., 1989). Minamishima et al. (2002) report that in 8−12 week old female mice before partial hepatectomy there were 78.6% 2C, 19.1% 4C, and 2.3% 8C cells but 7 days after there were 42.0% 2C, 49.1% 4C, and 9.0% 8C. Zajicek et al. (1991) describe how hepatocyte streaming is affected after the rapid hepatocyte DNA synthesis that occurs after the mitogenic stimulus of a partial hepatectomy. These data are of relevance to findings of increased DNA synthesis and liver weight gain following toxic insults and disease states. Zajicek et al. (1991) suggest that following a mitogenic stimulus, not all DNA synthesizing cells do divide but accumulate newly formed DNA and turn polyploid (i.e., during the first 3 days after partial hepatectomy in rats 50% of synthesized DNA was accumulated) and that since the acinus increased 15% and cell density declined 10%, overall cell mass increased 5%. However, cell influx rose 1,300%. “In order to accommodate all these cells, the ‘acinus-tube’ ought to swell 13-fold, while in reality it increased only 5%” and that on day 3 “the liver remnant did not even double in its size.” Zajicek et al. conclude that apparently “cells were eliminated very rapidly, and may have even been sloughed off, since the number of apoptotic bodies was very low” and therefore, “partial hepatectomy triggers two processes: an acute process lasting about a week marked by massive and rapid cell turnover during which most newly formed hepatocytes are eliminated, probably sloughed off into the sinusoids; and a second more protracted process which served for liver mass restoration mainly by forming new acini." Thus, a mitogenic stimulus may induce increased ploidy and increased cell number as a result of increased DNA synthesis, and many of the rapidly expanding number of cells resulting from such stimulation are purged and therefore, do not participate in subsequent disease states of the liver.

Zajicek et al. (1989) note that the accumulation of DNA rather than proliferation of hepatocytes "should be considered when evaluating the labeling index of hepatocytes labeled with tritiated thymidine" as the labeling index, defined as the proportion of labeled cells, can serve as a proliferation estimate only if it is assumed that a synthesizing cell will ultimately divide. In tissues, such as the liver, "where cells also accumulate DNA, proliferation estimates based on this index may fail" (Zajicek et al., 1989). The tendency to accumulate DNA is also accompanied by a decreasing probability of a cell to proliferate, since young hepatocytes generally divide after synthesizing DNA while older cells prefer instead to accumulate DNA. However, polyploidy *per se* does not preclude cells from dividing (Zajicek et al., 1989). The ploidy level achieved by the cell, no matter how high, does not, in itself, prevent it from going through the next mitotic cycle and the reproduction of hepatocytes in the ploidy classes of 8n and 8n X 2 is common phenomenon (Brodsky and Uryvaeva, 1977). However, along with a reduced capacity to proliferate, Sigal et al. (1999) report that the onset of polyploidy increases the probability of cell death. The proliferative potentials of hepatocytes not only depend on their ploidy, but also on the age of the animals with liver restoration occurring more slowly in aged animals after partial hepatectomy (Brodsky and Uryvaeva, 1977). Species differences in the ability of hepatocytes to proliferate and respond to a mitogenic stimulus have also been documented (see Section E.3.4.2, below). The importance of the issues of cellular proliferation versus DNA accumulation and the differences in ability to respond to a mitogenic stimulus becomes apparent as identification of the cellular targets of toxicity (i.e., diploid vs. polyploidy) and the role of proliferation in proposed MOAs are brought forth. Polyploidization, as discussed above, has been associated with a number of types of toxic injury, disease states, and carcinogenesis by a variety of agents.

E.1.2. Effects of Environment and Age: Variability of Response

The extent of polyploidization of the liver not only changes with age, but structural and functional changes, as well as environmental factors (e.g., polypharmacy), affect the vulnerability of the liver to toxic insult. In a recent review by Schmucker (2005), several of these factors are discussed. Schmucker reports that approximately 13% of the population of the United States is over the age of 65 years, that the number will increase substantially over the next

50 years, and that increased age is associated with an overall decline in health and vitality contributing to the consumption of nearly 40% of all drugs by the elderly. Schmucker estimates that 65% of this population is medicated and many are on polypharmacy regimes with a major consequence of a marked increase in the incidence of adverse drug reactions (ADRs) (i.e., males and females exhibit 3- and 4-fold increases in ADRs, respectively, when 20- and 60-year-old groups are compared). The percentage of deaths attributed to liver diseases dramatically increases in humans beyond the age of 45 years with data from California demonstrating a 4-fold increase in liver disease-related mortality in both men and women between the ages of 45 and 85 years (Seigel and Kasmin, 1997). Furthermore, Schmucker cites statistics from the United Stated Department of Health and Human Services to illustrate a loss in potential lifespan prior to 75 years of age due to liver disease (i.e., liver disease reduced lifespan to a greater extent than colorectal and prostatic cancers, to a similar extent as chronic obstructive pulmonary disease, and nearly as much as HIV). Thus, the elderly are predisposed to liver disease.

As stated above, the presence of high polyploidy cell in normal adults, nuclear polyploidization with age, and increase in the mean nuclear volume have been reported in people. Wantanabe et al. (1978) reported the results from a cytophotometrical analysis of 35 cases of sudden death including 22 persons over 60 years of age that revealed that although the nuclear size of most hepatocytes in a senile liver remains unchanged, there was an increase in cells with larger nuclei. Variations in both cellular area and nucleocytoplasmic ratio were also analyzed in the study, but the binuclearity of hepatocytes was not considered. No cases with a clinical history of liver disease were included. Common changes in senile liver were reported to include atrophy, fatty metamorphosis of hepatocytes, and occasional collapse of cellular cords in the centrilobular area, slight cellular infiltration and proliferation of Kupffer cells in sinusoids, and elongation of Glisson's triads with slight to moderate fibrosis in association with round cell infiltration. Furthermore, cells with giant nuclei, with each containing two or more prominent nucleoli, and binuclear cell. There was a decrease in diploid populations with age and an increase in tetraploid population and a tendency of polyploidy cells with higher values than hexaploids with age. Cells with greater nuclear size and cellular sizes were observed in livers with greater degrees of atrophy.

Schmucker notes that one of the most documented age-related changes in the liver is a decline in organ volume but also cites a decrease in functional hepatocytes and that other studies have suggested that the size or volume of the liver lobule increases as a function of increasing age. Data are cited for rats suggesting sinusoidal perfusion rate in the rat liver remains stable throughout the lifespan (Vollmar et al., 2002) but evidence in humans shows age-related shifts in the hepatic microcirculation attributable to changes in the sinusoidal endothelium (McLean et al.,

2003) (i.e., a 60% thickening of the endothelial cell lining and an 80% decline in the number of endothelial cell fenestrations, or pores, with increasing age in humans) that are similar in baboon liver (Cogger et al., 2003). Such changes could impair sinusoidal blood flow and hepatic perfusion, and the uptake of macromolecules such as lipoproteins from the blood. Schmucker reports that there is a consensus that hepatic volume and blood flow decline with increasing age in humans but that the effects of aging on hepatocyte structure are less clear. In rats, the volume of individual hepatocytes was reported to increase by 60% during development and maturation, but subsequently decline during senescence yielding hepatocytes of equivalent volumes in senescent and very young animals (Schmucker, 2005). The smooth surfaced endoplasmic reticulum (SER), which is the site of a variety of enzymes involved in steroid, xenobiotic, lipid and carbohydrate metabolism, also demonstrated a marked age-related decline rat hepatocytes (Schumucker et al., 1977, 1978). Schmucker also notes that several studies have reported that the older rodents have less effective protection against oxidative injury in comparison to the young animals, age-related decline in DNA base excision repair, and increases in the level of oxidatively damaged DNA in the livers of senescent animals in comparison to young animals. Age-related increases in the expression an activity of stress-induced transcription factors (i.e., increased NF-κB binding activity but not expression) were also noted, but that the importance of changes in gene expression to the role of oxidative stress in the aging process remains unsolved. An age-related decline in the proliferative response of rat hepatocytes to growth factors following partial hepatectomy was noted, but despite a slower rate of hepatic regeneration, older livers eventually achieved their original volume with the mechanism responsible for the age-related decline in the posthepatectomy hepatocyte proliferative response unidentified. As with other tissues, telomere length has been identified as a critical factor in cellular aging with the sequential shortening of telomeres to be a normal process that occurs during cell replication (see Sections E.3.1.1 and E.3.1.7, below). An association in telomere length and strain susceptibility for carcinogenesis in mice has been raised. Herrera et al., (1999) examined susceptibility to disease with telomere shortening in mice. However, this study only cites shorter telomeres for C57BL6 mice in comparison to mixed C57BL6/129sv mice. The actual data are not in this paper and no other strains are cited. Of the differing cell types examined, Takubo and Kaminishi (2001) report that hepatocytes exhibited the next fastest rate of telomere shortening despite being relatively long-lived cells raising the question of whether or not there are correlations between age, hepatocyte telomere length and the incidence of liver disease (Schmucker, 2005). Aikata et al. (2000) and Takubo et al. (2000) report that the mean telomere length in healthy livers is approximately 10 kilobase pairs at 80 years of age and these hepatocytes retain their proliferative capacity but that in diseased livers of elderly subjects was approximately 5 kb pairs. Thus, short telomere length may compromise hepatic regeneration and contribute to a poor prognosis in liver disease or as a donor liver (Schmucker, 2005).

Schmucker (2005) reports that interindividual variability in Phase I drug metabolism was so large in human liver microsomes, particularly among older subjects, that the determination of any statistically significant age or gender-related differences were precluded. In fact Schmucker (2001) notes that "the most remarkable characteristic of liver function in the elderly is the increase in interindividual variability, a feature that may obscure age-related differences." Schumer notes that The National Institute on Aging estimates that only 15% of individuals aged over 65 years exhibit no disease or disability with this percentage diminishing to 11 and 5% for men and women respectively over 80 years. Thus, the large variability in response and the presence of age-related increases in pharmacological exposures and disease processes are important considerations in predicting potential risk from environmental exposures.

E.2. CHARACTERIZATION OF HAZARD FROM TRICHLOROETHYLENE (TCE) STUDIES

The 2001 Draft assessment of the health risk assessment of TCE (U.S. EPA, 2001) extensively cited the review article by Bull (2000) to describe the liver toxicity associated with TCE exposure in rodent models. Most of the attention has been paid to the study of TCE metabolites, rather than the parent compound, and the review of the TCE studies by Bull (2000) was cursory. In addition, gavage exposure to TCE has been associated with a significant occurrence of gavage-related accidental deaths and vehicle effects, and TCE exposure through drinking water has been reported to decrease palatability and drinking water consumption, and to have significant loss of TCE through volatilization, thus, further limiting the TCE database. In its review of the draft assessment, U.S. Environmental Protection Agency (U.S. EPA)'s Science Advisory regarding this topic suggested that in its revision, the studies of TCE should be more fully described and characterized, especially those studies considered to be key for the hazard assessment of TCE. Although the database for studies of the parent compound is somewhat limited, a careful review of the rodent studies involving TCE can bring to bring to light the consistency of observations across these studies, and help inform many of the questions regarding potential MOAs of TCE toxicity in the liver. Such information can inform current MOA hypothesis (e.g., such as peroxisome proliferator activated receptor alpha [PPARα] activation) as well. Accordingly the primary acute, subchronic and chronic studies of TCE will be described and examined in detail below and with comments on consistency, major conclusions and the limitations and uncertainties that their design and conduct. Since all chronic studies were conducted primarily with the goal of ascertaining carcinogenicity, their descriptions focus on that endpoint, however, any noncancer endpoints described by the studies are described as well. For details regarding evidence of hepatotoxicity in humans and associations with increased risk of hepatocellular carcinoma, please refer to Sections 4.5.1 and 4.5.2. Given that some of the earlier studies with TCE were contaminated with epichlorhydrin, only the ones without such contamination are examined below.

E.2.1. Acute Toxicity Studies

A number of acute studies have been undertaken to describe the early changes in the liver after TCE administration with the majority using the oral gavage route of administration. Some have been detailed examinations while others have reported primarily liver weight changes as a marker of TCE-response. The matching and recording of age but especially initial and final body weight for control and treatment groups is of particular importance for studies using liver weight gain as a measure of TCE-response as difference in these parameter affect TCE-induced liver weight gain. Most data are for exposures of at least 10 days.

E.2.1.1. *Soni et al., 1998*

Soni et al. (1998) administered TCE in corn oil to male Sprague-Dawley (S-D) rats (200−250 g, 8−10 weeks old) intraperitoneally at exposure levels of 250, 500, 1,250, and 2,500 mg/kg. Groups (4−6 animals per group) were sacrificed at 0, 6, 12, 24, 36, 48, 72, and 96 hours after administration of TCE or corn oil. Using this paradigm only 50% of rats survived the 2,400 mg/kg intraperitoneal (i.p.) TCE administration with all deaths occurring between days 1 and 3 after TCE administration. Tritiated thymidine was also administered i.p. to rats 2 hours prior to euthanasia. Light microscopic sections of the central lobe in 3−4 sections examined for each animal. The grading scheme reported by the authors was: 0, no necrosis; +1 minimal, defined as only occasional necrotic cells in any lobule; +2, mild, defined as less than one-third of the lobular structure affected; +3, moderate, defined as between one-third and two-thirds of the lobular structure affected; +4 severe, defined as greater than two-thirds of the lobular structure affected. At the 2,500 mg/kg dose histopathology data were obtained for the surviving rats (50%). Lethality studies were done separately in groups of 10 rats. The survival in the groups of rats administered TCE and sacrificed from 0 to 96 hours was given as 30% mortality at 48 hours and 50% mortality by 72 hours.

The authors report that controls and 0-hour groups did not show sign of tissue injury or abnormality. The authors only report a single number with one significant figure for each group of animals with no means or standard deviations provided. In terms of the extent of necrosis there is no difference between the 250 and 500 mg/kg/treated dose groups though 96 hours with a single +1 given as the maximal amount of hepatocellular necrosis (minimal as defined by occasional necrotic cells in any lobule). At the 1,250 mg/kg dose the maximal score was achieved 24 hours after TCE administration and was reported as simply +2 (mild, defined as less than one-third of lobular structure affected). The level of necrosis was reported to diminish to a score of 0 72 hours after 250 mg/kg TCE with no decrease at 500 mg/kg. At 1,250 mg/kg, the extent of necrosis was reported to diminish from +2 to +1 by 72 hours after administration. At the 2,500 mg/kg dose (LD_{50} for this route) by 48 hours, the surviving rats were reported to have a score of +4 (severe as defined by greater than two thirds of the lobular structure affected). The authors report that

> The necrosed cells were concentrated mostly in the midzonal areas and the cells around central vein area were unaffected. Extensive necrosis was observed between 24 and 48 hours for both 1250 and 2500 mg/kg groups. Injury was maximal in the group receiving 2500 mg/kg between 36 and 48 hours as evidenced by severe midzonal necrosis, vacuolization, and congestion. Infiltration of polymorphonuclear cell was evident at this time as a mechanism for cleaning dead cells and tissue debris from the lobules. At the highest dose, the injury also started to spread toward the centrilobular areas. At highest dose, 30 and 50% lethality was observed at 48 and 72 h, respectively. After 48 h, the number of necrotic cells decreased and the number of mitotic cells increased. The groups receiving 500 and 1250 mg/kg TCE showed relatively higher mitotic activity as evidenced by cells in metaphase compared to other groups.

The authors do not give a quantitative estimate or indication as to the magnitude of the number of cells going through mitosis. Although there was variability in the number of animals dying at 1,250 mg/kg TCE exposure though this route of exposure, no indication of variability in response within these treatment groups is given by the author in regard to extent of histopathological changes. The authors do not comment on the manner of death using this paradigm or of the effects of i.p. administration regarding potential peritonitis and inflammation.

TCE hepatotoxicity was "assessed by measuring plasma" sorbitol dehydrogenase (SDH) and alanine aminotransferase (ALT) after TCE administration with vehicle treated control groups reported to induce no increases in these enzymes. Plasma SDH levels were reported to increase in a linear fashion after 250, 500, and 1,250 mg TCE/kg i.p. administration by 6 hours (i.e., ~3-, 10.5-, 22-, and 24.5-fold in comparison to controls from 250, 500, 1,250, and 2,500 mg/kg TCE, respectively) with little difference between the 1,250 and 250 mg/kg dose. By 12 hours the 250, 500, and 1,250 levels has diminished to levels similar to that of the 250 mg/kg dose at 6 hours. The 2,500 mg/kg levels was somewhat diminished from its 6 hour level. By 24 hours after TCE administration by the i.p. route of administration all doses were similar to that of the 250-mg/kg-

TCE 6-hour level. This pattern was reported to be similar for 5-, 36-, 48-, 72-, and 96-hour time points as well. The results presented were the means and SE for four rats per group. The authors did not indicate which rats were selected for these results from the 4−6 that were exposed in each group. Thus, only SDH levels showed dose dependence in results at the 6 hour time point and such increases did not parallel the patterns reported for hepatocellular necrosis from histopathological examination of liver tissues.

For ALT, the pattern of plasma concentrations after i.p. TCE administration differed both from that of SDH but also from liver histopathology. Plasma ALT levels were reported to increase in a nonlinear fashion and to a much smaller extent that SDH (i.e., ~2.7-, 1.9-, 2.1-, and 4.0-fold of controls from 250, 500, 1,250, and 2,500 mg/kg TCE, respectively). The patterns for 12, 24, 36, 48, 72, and 96 hours were similar to that of the 6-hour exposure and did not show a dose-response. The authors injected carbon tetrachloride (2.5.mL/kg) into a separate group of rats and then incubated the resulting plasma with unbuffered trichloroacetic acid (TCA; 0, 200, 600, or 600 nmol) and no decreases in enzyme activity *in vitro* at the two higher concentrations. It is not clear whether *in vitro* unbuffered TCE concentrations of this magnitude, which could precipitate proteins and render the enzymes inactive, are relevant to the patterns observed in the *in vivo* data. The extent of extinguishing of SDH and ALT activity at the two highest TCA levels *in vitro* were the same, suggestive of the generalized *in vitro* pH effect. However, the enzyme activity levels after TCE exposure had different patterns, and thus, suggesting that *in vitro* TCA results are not representative of the *in vivo* TCE results. Neither ALT nor SDH levels corresponded to time course or dose-response reported for the histopathology of the liver presented in this study.

Tritiated thymidine results from isolated nuclei in the liver did not show a pattern consistent with either the histopathology or enzyme results. These results were for whole-liver homogenates and not separated by nuclear size or cell origin. Tritiated thymidine incorporation was assumed by the authors to represent liver regeneration. There was no difference between treated and control animals at 6 hours after i.p. TCE exposure and only a decrease (~50% decrease) in thymidine incorporation after 12 hours of the 2,500 mg/kg TCE exposure level. By 24 hours, there as 5.6- and 2.8-fold tritiated thymidine incorporation at the 500 and 1,250 mg/kg TCE levels with the 250 and 2,500 mg/kg levels similar to controls. For 36, 48, and 72 hours after i.p. TCE exposure there continued to be no dose-response and no consistent pattern with enzyme or histopathological lesion patterns. The authors presented "area under the curve" data for tritiated thymidine incorporation for 0 to 95 hours, which did not include control values. There was a slight elevation at 500 mg/kg TCE and slight decrease at 2,500 mg/kg from the

250 mg/kg TCE levels. Again, these data did not fit either histopathology or enzyme patterns and also can include the contribution of nonparenchymal cell nuclei as well as changes in ploidy.

The use of an i.p. route of administration is difficult to compare to oral and inhalation routes of exposure given that peritonitis and direct contact with TCE and corn oil with liver surfaces may alter results. Whereas Soni et al. (1998) report the LD_{50} to be 2,500 mg/kg TCE via i.p. administration, both Elcombe et al. (1985) and Melnick et al. (1987) do not report lethality from TCE administered for 10 days at 1,500 mg/kg in corn oil, or up to 4,800 mg/kg/d for 10-days in encapsulated feed. Also TCE administered via gavage or oral administration through feed will enter the liver through the circulation with periportal areas of the liver the first areas exposed with the entire liver exposed in a fashion dependent on blood concentrations levels. However, with i.p. administration, the absorption and distribution pattern of TCE will differ. The lack of concordance with measures of liver toxicity from this study and the lack concordance of patterns and dose-response relationships of toxicity reported from other more environmentally and physiologically relevant routes of exposure make the relevance of these results questionable.

E.2.1.2. *Soni et al., 1999*

A similar paradigm and the same results were reported for Soni et al. (1999), in which hepatocellular necrosis, tritiated thymidine incorporation, and *in vitro* inhibition of SDH and ALT data were presented along with dose-response studies with ally alcohol and a mixture of TCE, Thioacetamine, allyl alcohol, and chloroform. The same issues with interpretation present for Soni et al. (1998) also apply to this study as well.

E.2.1.3. *Okino et al., 1991*

This study treated adult Wistar male rats (8 weeks of age) with TCE after being on a liquid diet for 3 weeks and either untreated or pretreated with phenobarbital or ethanol. TCE exposure was at 8,000 ppm for 2 hours, 2,000 or 8,000 ppm for 2 hours, and 500 or 2,000 ppm for 8 hours. Each group contained 5 rats. Livers from rats that were not pretreated with either ethanol or phenobarbital were reported to show only a few necrotic hepatocytes around the central vein at 6 and 22 hours after 2 hours of 8,000-ppm TCE exposure. At increased lengths and/or concentrations of TCE exposure, the frequencies of necrotic hepatocytes in the centrilobular area were reported to be increased but the number of necrotic hepatocytes was still relatively low (out of ~150 hepatocytes the percentages of necrotic pericentral hepatocytes were 0.2% ± 0.4%, 0.3% ± 0.4%, 2.7% ± 1.0%, 0.2% ± 0.4%, and 3.5% ± 0.4% for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively). "Ballooned" hepatocytes were reported to be zero for controls and all TCE treatments with the exception of 0.3% ± 0.6% ballooned midzonal hepatocytes after 8,000 ppm TCE for 2 hours exposure. Microsomal protein (mg/g/liver) was increased with TCE exposure concentration and duration, but not reported to be statistically significant (mg/g/liver microsomal protein was 21.2 ± 4.3, 22.0 ± 1.5, 25.9 ± 1.3, 23.3 ± 0.8, and 24.1 ± 1.0 for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively). The metabolic rate of TCE was reported to be increased after exposures over 2,000 ppm TCE (metabolic rate of TCE in nmol/g/liver/min was 29.5 ± 5.7, 51.3 ± 6.0, 63.1 ± 16.0, 37.3 ± 3.3, and 69.5 ± 4.3 for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively). However, the cytochrome P450 content of the liver was not reported to increase with TCE exposure concentration or duration. The liver/body weight ratios were reported to increase with all TCE exposures except 500 ppm for 8 hours (the liver/body weight ratio was 3.18% ± 0.15%, 3.35% ± 0.10%, 3.39% ± 0.20%, 3.15% ± 0.10%, and 3.57% ± 0.14% for control, 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours, respectively). These values represent 1.05-, 0.99-, 1.06-, and 1.12-fold of control in the 2,000 ppm TCE for 2 hours, 8,000 ppm TCE for 2 hours, 500 ppm TCE for 8 hours, and 2,000 ppm TCE for 8 hours treatment groups, respectively, with a statistically significant difference observed after 8 hours of 2,000-ppm TCE exposure. Initial body weights and those 22 hours after cessation of exposure were not reported, which may have affected liver weight gain. However, these data suggest that TCE-related increases in metabolism and liver weight occurred as early as 22 hours after exposures of this magnitude from 2 to 8 hours of TCE with little concurrent hepatic necrosis.

Ethanol and phenobarbital pretreatment were reported to enhance TCE toxicity. In ethanol-treated rats a few necrotic hepatocytes were reported to be around the central vein along with hepatocellular swelling without pyknotic nuclei at 6 hours after TCE exposure with no pathological findings in the midzonal or periportal areas. At 22 hours centrilobular hepatocytes were reported to have a few necrotic hepatocytes and cell infiltrations around the central vein but midzonal areas were reported to have ballooned hepatocytes with pyknotic nuclei frequently accompanied by cell infiltrations. In phenobarbital treated rats 6 hours after TCE exposure, centrilobular hepatocytes showed prenecrotic changes with no pathological changes reported to be observed in the periportal areas. By 22 hours, zonal necrosis was reported in centrilobular areas or in the transition zone between centrilobular and periportal areas. Treatment with phenobarbital or ethanol induced hepatocellular necrosis primarily in centrilobular areas with phenobarbital having a greater effect (89.1% ± 8.5% centrilobular necrosis) at the higher dose and shorter exposure duration (8,000 ppm × 2 hours) and ethanol having a greater effect (16.8% ± 5.3% centrilobular necrosis) at the lower concentration and longer duration of exposure (2,000 ppm × 8 hours).

E.2.1.4. *Nunes et al., 2001*

This study was focused on the effects of TCE and lead coexposure but treated male 75-day old S-D rats with 2,000 mg/kg TCE for 7 days via corn-oil gavage ($n = 10$). The rats ranged in weight from 293 to 330 g (~12%) at the beginning of treatment and were pretreated with corn oil for 9 days prior to TCE exposure. TCE was reported to be 99.9% pure. Although the methods section states that rats were exposed to TCE for 7 days, Table 1 of the study reports that TCE exposure was for 9 days. The beginning body weights were not reported specifically for control and treatment groups, but the body weights at the end of exposure were reported to be 342 ± 18 g for control rats and 323 ± 3 g for TCE exposed rats, and that difference (~6%) to be statistically significant. Because beginning body weights were not reported, it is difficult to distinguish whether differences in body weight after TCE treatment were treatment related or reflected differences in initial body weights. The liver weights were reported to be 12.7 ± 1.0 g in control rats and 14.0 ± 0.8 g for TCE treated rats with the percent liver/body weight ratios of 3.7 and 4.3%, respectively. The increase in percent liver/body weight ratio represents 1.16-fold of control and was reported to be statistically significant. However, difference in initial body weight could have affected the magnitude of difference in liver weight between control and treatment groups. The authors report no gross pathological changes in rats gavaged with corn oil or with corn oil plus TCE but observed that one animal in each group had slightly discolored brown kidneys. Histological examinations of "selected tissues" were reported to show an increased incidence of chronic inflammation in the arterial wall of lungs from TCE-dosed animals. There were no descriptions of liver histology given in this report for TCE-exposed animals or corn-oil controls.

E.2.1.5. *Tao et al., 2000*

The focus of this study was to assess the affects of methionine on methylation and expression of c-Jun and C-Myc in mouse liver after 5 days of exposure to TCE (1,000 mg/kg in corn oil) and its metabolites. Female 8-week old B6C3F1 mice ($n = 4-6$) were administered TCE ("molecular biology or HPLC grade") for 5 days with and without methionine (300 mg/kg i.p.). Data regarding % liver/body weight was presented as a figure. Of note is the decrease in liver/body weight ratio by methionine treatment alone (~4.6% liver/body weight for control and ~4.0% liver/body weight for control mice with methionine or ~13% difference between these groups). Neither initial body weights nor body weights after exposure were reported by the authors so that the reported effects of treatment could have reflected differences in initial body weights of the mice. TCE exposure was reported to increase the percent liver/body weight ratio to ~5.8% without methionine and to increase percent liver/body weight ratio to ~5.7% with methionine treatment. These values represent 1.26-fold of control levels from TCE exposure without methionine and 1.43-fold of control from TCE exposure with methionine. The number of animals examined was reported to be 4−6 per group. The authors reported the differences between TCE treated animals and their respective controls to be statistically significant but did not examine the differences between controls with and without methionine. There were no descriptions of liver histology given in this report for TCE-exposed animals or corn-oil controls.

E.2.1.6. *Tucker et al., 1982*

This study describes acute LD_{50}, and 5- and 14-days studies of TCE in a 10% emulphor solution administered by gavage. Screening level subchronic drinking water experiments with TCE dissolved in 1% emulphor in mice were also conducted but with little detail reported. The authors did describe the strains used (CD-1 and ICR outbred albino) and that they are "weanling mice," but the ages of the mice and their weights were not given. The TCE was described as containing 0.004% diisopropylamine as the preservative and that the stabilizer had not been found carcinogenic or overtly toxic. The authors report that "the highest concentration a mouse would receive during these studies is 0.03 mg/kg/day." The main results are basically an LD_{50} study and a short term study with limited reporting for 4 and 6-month studies of TCE. Importantly, the authors documented the loss of TCE from drinking water solutions (less than 20% of the TCE was lost during the 3 or 4 days in the water bottles at 1.0, 2.5, and 5.0 mg/mL concentrations, but in the case of 0.1 mg/mL, up to 45% was lost over a 4-day period). The authors also report that high doses of TCE in drinking water reduced palatability to such an extent that water consumption by the mice was significantly decreased.

The LD_{50} with 95% confidence were reported to be 2,443 mg/kg (1,839 to 3,779) for female mice and 2,402 mg/kg (2,065 to 2,771) for male mice. However, the number of mice used in each dosing group was not given by the authors. The deaths occurred within 24 hours of TCE administration and no animals recovering from the initial anesthetic effect of TCE died during the 14-day observation period. The authors reported that the only gross pathology observed was hyperermia of the stomach of mice dying form lethal doses of TCE, and that mice killed at 14 days showed not gross pathology. In a separate experiment, male CD-1 mice were exposed to TCE by daily gavage for 14 days at 240 and 24 mg/kg. These two doses did not cause treatment related deaths and body weight and "most" organ weights were reported by the authors to not be significantly affected but the data was not shown. The only effect noted was an increased liver weight, which appeared to be dose dependent but was reported to be significant only at the higher dose. The only significant difference found in hematology was s 5% lower hematocrit in the higher dose group. The number of animals tested in this experiment was not give by the authors. Male CD-1 mice (*n* = 11) were given TCE via gavage for 5 days (0.73 g/kg TCE twice on Day 0, 1.46 g/kg twice on Day 1, 2.91 g/kg twice on Day 3, and 1.46 g/kg TCE on Days 4 and 5) with only 4 of 11 mice treated with TCE surviving.

In a subchronic study, male and female CD-1 mice received TCE in drinking water at concentrations of 0, 0.1, 1.0, 2.5, and 5 mg/mL in 1% emulphor, and a naïve group received deionized water. There were 140 animals of each sex in the naïve group and in each treatment group, except for 260 mice in the vehicle groups. Thirty mice of each sex and treatment were selected for recording body weights for 6 months. The method of "selection" was not given by the authors. These mice were weighed twice weekly and fluid consumption was measured by weighing the six corresponding water bottles. The authors reported that male mice at the two highest doses of TCE consumed 41 and 66 mL/kg/day less fluid over the 6 months of the study than mice consuming vehicle only and that this same decreased consumption was also seen in the high dose (5 mg/mL) females. They report that weight gain was not affected except at the high dose (5mg/mL) and even though the weight gain for both sexes was lower than the vehicle control group, it was not statistically significant but these data were not shown. The authors report that gross pathological examinations performed on mice killed at 4 and 6 months were unremarkable and that a number of mice from all the dosing regimens had liver abnormalities, such as pale, spotty, or granular livers. They report that 2 of 58 males at 4 months, and 11 of 59 mice at 6 months had granular livers and obvious fatty infiltration, and that mice of both sexes were affected. Animals in the naïve and vehicle groups were reported to infrequently have pale or spotty livers, but exhibit no other observable abnormalities. No quantitation or more detailed descriptions of the incidence of or severity of effects were given in this report.

The average body weight of male mice receiving the highest dose of TCE was reported to be 10% lower at 4 months and 11% lower at 6 months with body weights of female mice at the highest dose also significantly lower. Enlarged livers (as percentage of body weight) were observed after both durations of exposure in males at the three highest doses, and in females at the highest dose. In the 4-month study, brain weights of treated females were significantly increased when compared to vehicle control. However, the authors state

> this increase is apparently because the values for the vehicle group were low, because the naïve group was also significantly increased when compared to vehicle control. A significant increase in kidney weight occurred at the highest

> dose in males at 6 months and in females, after both 4 and 6 months of TCE exposure. Urinalysis indicated elevated protein and ketone levels in high-dose females and the two highest dose males after 6 months of exposure (data not shown).

The authors describe differences in hematology to include

> a decreased erythrocyte count in the high dose males at 4 and 6 months (13% and 16%, respectively); decreased leukocyte counts, particularly in the females at 4 months and altered coagulation values consisting of increased fibrinogen in males at both times and shortened prothrombin time in females at 6 months (data not shown). No treatment-related effects were detected on the types of white cells in peripheral blood.

It must be noted that effects reported from this study may have also been related to decreased water consumption, this study did included any light microscopic evaluation, and that most of the results described are for data not shown. However, this study does illustrate the difficulties involved in trying to conduct studies of TCE in drinking water, that the LD_{50}s for TCE are relatively high, and that liver weight increases were observed with TCE exposure as early as few weeks and increased liver weight were sustained through the 6-month study period.

E.2.1.7. *Goldsworthy and Popp, 1987*

The focus of this study was peroxisomal proliferation activity after exposure to a number of chlorinated solvents. In this study 1,000 mg/kg TCE (99+ % epoxide stabilizer free) was administered to male F-344 rats (170−200 g or ~10% difference) and B6C3F1 (20−25 g or ~20% difference) mice for 10 days in corn oil via gavage. The ages of the animals were not given. The TCE-exposed animals were studied in two experiments (Experiments #1 and #3). In experiment #2 corn oil and methyl cellulose vehicles were compared. Animals were killed 24 hours after the last exposure. The authors did not show data on body weight but stated that the administration of test agents (except WY-14,643 to rats which demonstrated no body weight gain) to rats and mice for 10 days "had little or no effect on body weight gain." Thus, differences in initial body weight between treatment and control groups, which could have affected the magnitude of TCE-induced liver weight gain, were not reported. The liver/body weight ratios in corn oil gavaged rats were reported to be 3.68% ± 0.06% and 4.52% ± 0.08% after TCE treatment which represented 1.22-fold of control (n = 5). Cyanide-(CN-)insenstive palmitoyl CoA[1] oxidation (PCO) was reported to be 1.8-fold increased after TCE treatment in this same group. In B6C3F1 mice the

[1]CoA = coenzyme A.

liver/body weight ratio in corn oil gavaged mice was reported to be 4.55% ± 0.13% and 6.83% ± 0.13% after TCE treatment which represented 1.50-fold of control (*n* = 7). CN-insensitive PCO activity was reported to be 6.25-fold of control after TCE treatment in this same group. The authors report no effect of vehicle on PCO activity but do not show the data nor discuss any effects of vehicle on liver weight gain. Similarly the results for experiment #3 were not shown nor liver weight discussed with the exception of PCO activity reported to be 2.39-fold of control in rat liver and 6.25-fold of control for mouse liver after TCE exposure. The number of animals examined in Experiment #3 was not given by the authors or the variation between enzyme activities. However, there appeared to be a difference in PCO activity Experiments #1 and #3 in rats. There were no descriptions of liver histology given in this report for TCE-exposed animals or corn-oil controls.

E.2.1.8. *Elcombe et al., 1985*

In this study, preservative free TCE was given via gavage to rats and mice for 10 consecutive days with a focus on changes in liver weight, structure, and hepatocellular proliferation induced by TCE. Male Alderly Park rats (Wistar derived) (180−230 g), male Obsborne-Mendel rats (240−280 g), and male B6C3F1 or male Alderly Park Mice (Swiss) weighing 30 to 35 g were administered 99.9% pure TCE dissolved in corn oil via gavage. The ages of the animals were not given by the authors. The animals were exposed to 0, 500, 1,000, or 1,500 mg/kg body wt TCE for 10 consecutive days. The number of mice and rats varied widely between experiments and treatment groups and between various analyses. In some experiments animals were injected with tritiated thymidine approximately 24 hours following the final dose of TCE and killed one hour later. The number of hepatocytes undergoing mitosis was identified in 25 random high-power fields (X40) for each animal with 5,000 hepatocyte per animal examined. There was no indication by the authors that zonal differences in mitotic index were analyzed. Sections of the liver were examined by light and electron microscopy by conventional staining techniques. Tissues selected for electron microscopy included central vein and portal tract so that zonal differences could be elucidated. Morphometric analysis of peroxisomes was performed "according to general principles of Weibel et al (1964) on electronphotomicrographs from pericentral hepatocytes." DNA content of samples and peroxisomal enzyme activities were determined in homogenized liver (catalase and PCO activity).

The authors reported that TCE treatment had no significant effect on body-weight gain either strain of rat or mouse during the 10 days exposure period. However, marked increases (up to 175% of control value) in the percent liver/body weight ratio were observed in TCE-treated mice. Smaller increases (up to 130% of control) in relative liver weight were observed in TCE-treated rats. No significant effects of TCE on hepatic water content were seen so that the liver weight did not represent increased water retention.

An interesting feature of this study was that it was conducted in treatment blocks at separate times with separate control groups of mice for each experimental block. Therefore, there were three control groups of B6C3F1 mice (*n* = 10 for each control group) and three control groups for Alderly Park (*n* = 9 to 10 for each control group) mice that were studied concurrently with each TCE treatment group. However, the percent liver/body weight ratios were not the same between the respective control groups. There was no indication from the authors as to how controls were selected or matched with their respective experimental groups. The authors did not give liver weights for the animals so the actual changes in liver weights are not given. The body weights of the control and treated animals were also not given by the authors. Therefore, if there were differences in body weight between the control groups or treatment groups, the liver/body weight ratios could also have been affected by such differences. The percentage increase over control could also have been affected by what control group each treatment group was compared to. There was a difference in the mean percent liver/body weight ratio in the control groups, which ranged from 4.32 to 4.59% in the B6C3F1 mice (~6% difference) and from 5.12 to 5.44% in the Alderly Park mice (~6% difference). The difference in average percent liver/body weight ratio for untreated mice between the two strains was ~16%. Because the ages of the mice were not given, the apparent differences between strains may have been due to both age or to strain. After TCE exposure, the mean percent liver/body weight ratios were reported to be 5.53% for 500 mg/kg, 6.50% for 1,000 mg/kg, and 6.74% for 1,500 mg/kg TCE-exposed B6C3F1 mice. This resulted in 1.20-, 1.50-, and 1.47-fold values of control in percent liver weight/body weight for B6C3F1 mice. For Alderly Park mice, the percent liver/body weight ratios were reported to be 7.31, 8.50, and 9.54% for 500, 1,000, and 1,500 mg/kg TCE treatment, respectively. This resulted in 1.43-, 1.56-, and 1.75-fold of control values. Thus, there appeared to be more of a consistent dose-related increase in liver/body weight ratios in the Alderly Park mice than the B6C3F1 mice after TCE treatment. However, the variability in control values may have distorted the dose-response relationship in the B6C3F1 mice. The Standard deviations for liver/body weight ratio were as much as 0.52% for the treated B6C3F1 mice and 0.91% for the Alderly Park treated mice. In regard to the correspondence of the magnitude of the TCE-induced increases in percent liver/body weight with the magnitude of difference in TCE exposure concentrations, in the B6C3F1 mice the increases were similar (~2-fold) between the 500 mg/kg and 1,000 mg/k TCE exposure groups. For the Alderly Park mice, the increases in TCE exposure concentrations were slightly less than the magnitude of increases in percent liver/body ratios between all of the concentrations (i.e., ~1.3-fold of control vs. 2-fold for 500 and 1,000 mg/kg TCE dose and 1.3-fold of control vs. 1.5-fold for the 1,000 and 1,500 mg/kg TCE dose).

The DNA content of the liver varied greatly between control animal groups. For B6C3F1 mice it ranged from 2.71 to 2.91 mg/g liver. For Alderly Park mice it ranged from 1.57 to 2.76 mg/g liver. The authors do not discuss this large variability in baseline levels of DNA content. The DNA content in B6C3F1 mice was mildly depressed by TCE treatment in a nondose dependent manner. DNA concentration decrease from control ranged from 20−25% between all three TCE exposure levels in B6C3F1 mice. For Alderly Park mice there was also nondose related decrease in DNA content from controls that ranged from 18% to 34%. Thus, the extent of decrease in DNA content of the liver from TCE treatment in B6C3F1 mice was similar to the variability between control groups. The lack of dose-response in apparent treatment related effect in B6C3F1 mice and especially in the Alderly Park mice was confounded by the large variability in the control animals. The changes in liver weight after TCE exposure for the AP mice did not correlate with changes in DNA content further, raising doubt about the validity of the DNA content measures. However, a small difference in DNA content due to TCE treatment in all groups was reported for both strains and this is consistent with hepatocellular hypertrophy.

The reported results for incorporation of tritiated thymidine in liver DNA showed large variation in control groups and standard deviations that were especially evident in the Alderly Park mice. For B6C3F1 mice, mean control levels were reported to range from 5,559 to 7,767 dpm/mg DNA with standard deviations ranging from 1,268 to 1,645 dpm/mg DNA. In Alderly Park mice mean control levels were reported to range from 6,680 to 10,460 dpm/mg DNA with standard deviations ranging from 308 to 5,235 dpm/mg DNA. For B6C3F1 mice, TCE treatment was reported to induce an increase in tritiated thymidine incorporation with a very large standard deviation, indicating large variation between animals. For 500 mg/kg TCE treatment group the values were reported as 12,334 ± 4,038, for 1,000 mg/kg TCE treatment group 21,909 ± 13,386, and for 1,500 mg/kg treatment TCE group 26,583 ± 10,797 dpm/mg DNA. In Alderly Park mice TCE treatment was reported to give an increase in tritiated thymidine incorporation also with a very large standard deviation. For 500 mg/kg TCE, the values were reported as 19,315 ± 12,280, for 1,000 mg/kg TCE 21,197 ± 8,126 and for 1,500 mg/kg TCE 38,370 ± 13,961. As a percentage of concurrent control, the increase in tritiated thymidine was reported to be 2.11-, 2.82-, and 4.78-fold of control in B6C3F1 mice, and 2.09-, 2.03-, and 5.74-fold of control in Alderly Park mice. Accordingly, the change in tritiated thymidine incorporation did show a treatment related increase but not a dose-response. Similar to the DNA content of the liver, the large variability in measurements between control groups and variability between animals limit quantitative interpretation of these data. The increase in tritiated thymidine, seen most consistently only at the highest exposure level in both strains of mice, could have resulted from either a change in ploidy of the hepatocytes or cell number. However, the large change in volume in the liver (75%) in the Alderly Park mice, could not have resulted from only a 4-fold of control in cell proliferation even if all tritiated thymidine incorporation had resulted from changes in hepatocellular proliferation. As mentioned in Section E.1.1 above, the baseline level of hepatocellular proliferation in mature control mice is very low and represents a very small percentage of hepatocytes.

In the experiments with male rats, the same issues discussed above, associated with the experimental design, applied to the rat experiments with the additional concern that the numbers of animals examined varied greatly (i.e., 6 to 10) between the treatment groups. In Obsborne-Mendel rats, the control liver/body weight ratio was reported to vary from 4.26 to 4.36% with the standard deviations varying between 0.22 to 0.27%. For the Alderly Park rats, the liver/body weight ratios were reported to vary between 4.76 and 4.96% (in control groups) with standard deviations varying between 0.24 to 0.47%. TCE treatment was reported to induce a dose-related increase in liver/body weight ratio in Obsborne-Mendel rats with mean values of 5.16, 5.35, and 5.53% in 500, 1,000, and 1,500 mg/kg TCE treated groups, respectively. This resulted in 1.18-, 1.26-, and 1.30-fold values of control. In Alderly Park rats, TCE treatment was reported to result in increased liver weights of 5.45, 5.83, and 5.65% for 500, 1,000, and 1,500 mg/kg TCE respectively. This resulted in 1.14-, 1.17-, and 1.17-fold values of control. Again, the variability in control values may have distorted the nature of the dose-response relationships in Alderly Park rats. TCE treatment was reported to result in standard deviations that ranged from 0.31 to 0.48% for OM rats and 0.24 to 0.38% for Alderly Park rats. What is clear from these experiments is that TCE exposure was associated with increased liver/body weight in rats.

The reported mean hepatic DNA concentrations and standard deviations varied greatly in control rat liver as it did in mice. The variation in DNA concentration in the liver varied more between control groups than the changes induced by TCE treatment. For Obsborne-Mendel rats, the mean control levels of mg DNA/g liver were reported to range from 1.99 to 2.63 mg DNA/liver with standard deviations varying from 0.17 to 0.33 mg DNA/g. For Alderly Park rats, the mean control levels of mg DNA/g liver were reported to be 2.12 to 3.16 mg DNA/g with standard deviation ranging from 0.06 to 1.04 mg DNA/g. TCE treatment decreased the liver DNA concentration in all treatment groups. For Obsborne-Mendel rats, the decrease ranged from 8 to 13% from concurrent control values and for Alderly Park rats the decrease ranged from 8 to 17%. There was no apparent dose response in the decreases in DNA content with all TCE treatment levels giving a similar decrease from controls and the same limitations discussed above for the mouse data apply here. The magnitude of increases in liver/body ratios shown by TCE treatment were not correlated with the changes in DNA content. However, as with the mouse data, the small differences in DNA content due to TCE treatment in all groups and in both strains was consistent with hepatocellular hypertrophy.

Incorporation of tritiated thymidine was reported to be even more variable between control groups of rats than it was for mice and was reported to be especially variable between control groups (i.e., 2.7-fold difference between control groups within strain) and differed between the strains (average of 2.5-fold between strains). For Obsborne-Mendel rats the mean control levels were reported to range from 13,315 to 33,125 dpm/mg DNA, while for Alderly Park rats tritiated thymidine incorporation ranged from 26,613 to 69,331 dpm/mg DNA for controls. The standard deviations were also very large (i.e., for control groups of Obsborne-Mendel rats they were reported to range from 8,159 to 13,581 dpm/mg DNA, while for Alderly Park rats they ranged from 9,992 to 45,789 dpm/mg DNA). TCE treatment was reported to induce increases over controls of 110, 118, and 106% for 500, 1,000, and 1,500 mg/kg TCE-exposed groups, respectively, in Obsborne-Mendel rats with large standard deviations for these treatment groups as well. In Alderly Park rats, the increases over controls were reported to be 206, 140, and 105% for 500, 1,000, and 1,500 mg/kg TCE, respectively. In general, these data do indicate that TCE treatment appeared to give a mild increase in tritiated thymidine incorporation but the lack of dose-response can be attributable to the highly variable measurements of tritiated thymidine incorporation in control animal groups. The variation in the number of animals examined between groups and small numbers of animals examined additionally decrease the likelihood of being able to discern the magnitude of difference between species- or strain-related effects for this parameter. Again, given the very low level of hepatocyte turnover in control rats, this does not represent a large population of cells in the liver that may be undergoing proliferation and cannot be separated from changes in ploidy.

The authors report that the reversibility of these phenomena was examined after the administration of TCE to Alderly Park mice for 10 consecutive days. Effects upon liver weight, DNA concentration, and tritiated thymidine incorporation 24 and 48 hours after the last dose of TCE were reported to be still apparent. However, 6 days following the last dose of TCE, all of these parameters were reported to return to control values with the authors not showing the data to support this assertion. Thus, cessation of TCE exposure would have resulted in a 75% reduction in liver weight by one week in mice exposed to the highest TCE concentration.

Analyses of hepatic peroxisomal enzyme activities were reported for catalase and β-oxidation (PCO activity) following administration of TCE to B6C3F1 mice and Alderly Park rats exposed to 1,000 mg/kg TCE for 10 days. The authors only used 5 control and 5 exposed animals for these tests. An 8-fold of control value for PCO activity and a 1.5-fold of control value for catalase activity were reported for B6C3F1 mice exposed to 1,000 mg/kg TCE. In the Alderly Park rats no significant changed occurred. It is unclear which mice or rats were selected from the previous experiments for these analyses and what role selection bias may have played in these results. The reduced number of animals chosen for this analysis also reduces the power of the analysis to detect a change. In rats, there was a reported 13% increase in PCO; however, the variation between the TCE treated rats was more than double that of the control animals in this group and the other limitations described above limit the ability to detect a response. There was no discussion given by the authors as to why only one dose was tested in half of the animals exposed to TCE or why the strain with the lowest liver weight change due to TCE exposure was chosen as the strain to test for peroxisomal proliferative activity.

The authors provided a description of the histopathology at the light microscropy level in B6C3F1 mice, Alderly Park mice, Osborne-Mendel rats, and Alderly Park rats, but did not provide a quantitative analysis or specific information regarding the variability of response between animals within groups. There appeared to be 20 animals examined in the 1,000 mg/kg TCE exposed group of B6C3F1 mice but no explanation as to why there were only 10 animals examined in analyses for liver weight changes, DNA concentration, and tritiated thymidine incorporation. There was no indication by the authors regarding how many rats were examined by light microscopy.

Apart from a few inflammatory foci in occasional animals, hematoxylin and eoxin (H&E) section from B6C3F1 control mice were reported to show no abnormalities. The authors suggest that this is a normal finding in the livers of mice kept under "non-SPF conditions." A stain for neutral lipid was reported to not be included routinely in these studies, but subsequent electron microscopic examination of lipid to show increases in the livers of corn-oil treated control animals. The individual fat droplets were described as "generally extremely fine and are not therefore detectable in conventionally process H&E stained sections, since both glycogen and lipid are removed during this procedure." Thus, this study documents effects of using corn oil gavage in background levels of lipid accumulation in the liver.

The finding of little evidence of gross hepatotoxicity in TCE-treated mice was reported, even at a dose of 1,500 mg/kg. Specifically,

> Of 19 animals examined receiving 1500 mg/kg body weight TCE, only 6 showed any evidence of hepatocyte necrosis, and this pathology was restricted to single small foci or isolated single cells, frequently occurring in a subcapsular location. Examination of 20 animals receiving 1000 mg/kg body wt TCE demonstrated no

> hepatocyte necrosis. Of 20 animals examined receiving 500 mg/kg body wt TCE, 1 showed necrosis of single isolated hepatocytes; however, this change was not a treatment-related finding.

TCE-treated mice were reported to show

> a change in staining characteristic of the hepatocytes immediately adjacent to the central vein of the hepatocyte lobules, giving rise to a marked 'patchiness' of the liver sections. Often this change consisted of increased eosinophilia of the central cells. There was some evidence of cell hypertrophy in the centrilobular regions. These changes were evident in most of the TCE treated animals, but there was a dose-related trend, relatively few of the 500 mg/kg animals being affected, while the majority of the 1,500 mg/kg animals showed central change. No other significant abnormalities were seen in the liver of TCE treated mice compared to controls apart from occasional mitotic figures and the appearance of isolated nuclei with an unusual chromatin pattern. This pattern generally consisted of a course granular appearance with a prominent rim of chromatin around the periphery of the nucleus. These nuclei may have been in the very early stages of mitosis. Similar changes were not seen in control mice.

The authors briefly commented on the findings in the Alderly Park mice stating that

> H& E sections from Alderly Park mice gave similar results as for B6C3F1 mice. No evidence of hepatotoxicity was seen at a dose of 500 mg/kg body wt TCE. However, a few animals at the higher doses showed some necrosis and other degenerative changes. This change was very mild in nature, being restricted to isolated necrotic cells or small foci, frequently in subcapsular position. Hypertrophy and increased eosinophilia were also noticed in the centrilobular regions at higher doses.

Thus, from the brief description given by the authors, the centrilobular region is identified as the location of hepatocellular hypertrophy due to TCE exposure in mice, and for it to be dose-related with little evidence of accompanying hepatotoxicity.

The description of histopathology for rats was even more abbreviated than for the mouse. H& E sections from Osborne-Mendel rats showed that

> livers from control rats contained large quantities of glycogen and isolated inflammatory foci, but were otherwise normal. The majority of rats receiving 1,500 mg/kg body weight TCE showed slight changes in centrilobular hepatocytes. The hepatocytes were more eosinophilic and contained little glycogen. At lower doses, these effects were less marked and were restricted to fewer animals. No evidence of treatment-related hepatotoxicity (as exemplified

by single cell or focal necrosis) was seen in any rat receiving TCE. H& E sections from Alderly Park Rats showed no signs of treatment-related hepatotoxicity after administration of TCE. However, some signs of dose-related increase in centrilobular eosinophilia were noted.

Thus, both mice and rats exhibited pericentral hypertrophy and eosinophilia as noted from the histopathological examination.

The study did report a quantitative analysis of the effects of TCE on the number of mitotic figures in livers of mice. Few if any control mice exhibited mitotic figures. But, the authors report

> a considerable increase in both the numbers of figures per section was noted after administration of TCE." The numbers of animals examined for mitotic figures ranged from 75 (all control groups were pooled for mice) to 9 in mice, and ranged from 15 animals in control rat groups to as low as 5 animals in the TCE treatment groups. The range of mitotic figures found in 25 high-power fields was reported and is equivalent to the number of mitotic figures per 5,000 hepatocytes examined in random fields.

Thus, the predominance of mitotic figures in any zone of the liver cannot be ascertained.

For B6C3F1 mice the number of animals with mitotic figures was reported to be 0/75, 3/20, 7/20, and 5/20 for control, 500, 1,000, and 1,500 mg/kg TCE exposed mice, respectively. The range of the number of mitotic figures seen in 5,000 hepatocytes was reported to be 0, 0−1, 0−5, 0−5 for those same groups with group means of 0, 0.15 ± 0.36, 0.6 ± 1.1, and 0.5 ± 1.2. These results demonstrate a very small and highly variable response due to TCE treatment in B6C3F1 mice in regard to mitosis. Thus, the highest percentage of cells undergoing mitosis within the window of observation would be on average 0.012% with a standard deviation twice that value. The data presented for mitotic figures also indicated no differences in results between 1,000 and 1,500 mg/kg treated B6C3F1 mice in regard to mitotic figure detection. However, the tritiated thymidine incorporation data indicated that thymidine incorporation was ~2-fold greater at 1,500 than 1,000 mg/kg TCE in B6C3F1 mice. For Alderly Park mice, the number of animals with mitotic figures was reported to be 1/15, 0/9, 4/9, and 2/9 for control, 500, 1,000, and 1,500 mg/kg TCE exposed mice. The range of the number of mitotic figures seen in 5,000 hepatocytes was 0−1, 0, 0−2, 0−1 for those same groups with group means of 0.06 ± 0.25, 0.7 ± 0.9, and 0.2 ± 0.4. These results reveal the detection of at the most 2 mitotic figure in 5,000 hepatocytes for any mouse an any treatment group and no dose-related increased after TCE treatment in Alderly Park mice. Thus, the highest percentage of cells with a mitotic figure would be on average 0.014% with a standard deviation twice that value. The small number of animals examined reduces the power of the experiment to draw any conclusions as to a dose-response. Similar to the B6C3F1 mice, there did not appear to be concordance between mitotic figure detection and thymidine incorporation for Alderly park mice. Thymidine incorporation showed a 2-fold increase over control for 500 and 1,000 mg/kg TCE and a 5.7-fold increase for 1,500 mg/kg TCE treated animals. However, in regard to mitotic figure detection, there were fewer mitotic figures in 500 mg/kg TCE treated mice than controls, and fewer animals with mitotic figures and fewer numbers of figures in the 1,500 mg/kg dose than the 1,000 mg/kg exposed group. The inconsistencies between mitotic index data and thymidine incorporation data in both strains of mice suggests that either thymidine incorporation is representative of only DNA synthesis and not mitosis, an indication of changes in ploidy rather than proliferation, or that this experimental design is incapable of discerning the magnitude of these changes accurately. Data from both mouse strains show very little if any hepatocyte proliferation due to TCE exposure with the mitotic figure index data having that advantage of being specific for hepatocytes and to not to also include nonparenchymal cells or inflammatory cells in the liver.

The results for rats were similar to those for mice and even more limited by the varying and low number of animals examined. For Osborne-Mendal rats the number of animals with mitotic figures were reported to be 8/15, 2/9, 0/7, and 0/6 for control, 500, 1,000, and 1,500 mg/kg TCE exposed rats groups, respectively, with the range of the number of mitotic figures seen in 5,000 hepatocytes to be 0−8, 0−3, 0, and 0. The group mean was 1.5 ± 2.0, 0.4 ± 1.0, 0, and 0 for these groups. It would appear from these results that there are fewer mitotic figures after TCE treatment with the highest percentage of cells undergoing mitosis to be on average 0.03% in control rats. However, thymidine incorporation studies show a modest increase at all treatment levels over controls in Osborne Mendel rats rather than a decrease from controls. For Alderly Park rats the number of animals with mitotic figures was reported to be 13/15, 5/9, 9/9, and 4/9 for control, 500, 1,000, and 1,500 mg/kg TCE exposed rat groups with the range of the number of mitotic figures seen in 5,000 hepatocytes to be 0−26, 0−5, 1−7, and 0−9. The group mean was 7.2 ± 4.7, 1.6 ± 4.3, 3.8 ± 3.4, and 1.8 ± 2.9 for these groups. It would appear that there are fewer mitotic figures after TCE treatment with the highest percentage of cells to an average of 0.14% in control rats. However, thymidine incorporation studies show 2-fold greater level at 500 mg/kg TCE than for control animals and a 40 and 5% increase at 1,000 mg/kg and 1,500 mg/kg TCE exposure groups, respectively. Similar to the results reported in mice, results in both rat strains show an inconsistency in mitotic index and thymidine incorporation. The control rats appear to have a much greater mitotic index than any of the mouse groups (treated or untreated) or the TCE-treatment groups. However, it is the mice that were exhibiting the largest increased in liver weight after TCE exposure. By either thymidine incorporation or mitosis, these data do provide a consistent result that at 10 days of exposure very little sustained hepatocellular proliferation is occurring in either mouse or rat and neither is correlated well with the concurrent changes in liver weight observed from TCE exposure.

This study provided a qualitative discussion and quantitative analysis of structural changes using electron microscopy. The qualitative discussion was limited and included statements about increased observances without quantitative data shown other than the morphometric analysis. The authors reported that

> the ultrastructure of control mouse liver was essentially normal, although mild dilatation of RER and SER was a frequent finding. Lipid droplets were also usually present in the cell cytoplasm. The ultrastructural changes seen in mouse liver following administration of up to 1,500 mg/kg body wt TCE for 10 days were essentially similar in the B6C3F1 mouse and the Alderly Park mouse. The most notable change in both strains of mouse was a dramatic increase in the number of peroxisomes. This change was only apparent in the cells immediately surrounding the central veins. Peroxisome proliferation was not noticeable in periportal cells. The induced peroxisomes were generally small and very electron dense and frequently lacked the characteristic nucleoid core found in peroxisomes of control livers.

The authors conclude that

> morphometric analysis showed evidence of a dose-related response, peroxisomal induction appearing to reach a maximum at 1,000 mg/kg in B6C3F1 mice…Lipid was increased in the livers of treated mice at all doses and was present both as free droplets in the cytoplasm and as liposomes (small lipid droplets in ER cisternae). The centrilobular cell, which showed the greatest increase in numbers of peroxisomes, showed no evidence of this lipid accumulation: fatty change was more prominent in those cells away from the central vein (i.e., zone 2 of the liver acinus). Accumulation of lipid, particularly in liposomes, was less marked in Alderly Park mouse than in B6C3F1 mouse. Mild proliferation of smooth endoplasmic reticulum was seen in both strains and both rough and smooth endoplasmic reticulum was generally more dilated than in control mice.

Electron microscopic results for rat liver were reported

> to show similar changes in Osborne-Mendel and Alderly Park rat treated with TCE…Rats receiving either 1,000 or 1,500 mg/kg TCE for 10 days generally showed mild proliferation of SER in centrilobular hepatocytes. The cisternae of RER were frequently dilated, giving rise to a rather disorganized appearance in contrast to the parallel stacks seen in control livers, although no detachment of

> ribosomes was evident. The SER was also dilated. In contrast to mice, peroxisomes were only very slightly and not significantly, increased in the liver of TCE –treated rats. Morphometric analysis confirmed this observation, with the volume density of peroxisomes in the cytoplasm of centrilobular hepatocytes being only slightly increased in rats of both strains receiving 1,000 or 1,500 mg/kg body wt TCE…Lipid droplets were occasionally increased in some livers obtained from rats receiving TCE, but the degree of fatty change generally appeared similar to that found in control rats receiving corn oil. There were no changes in membrane –bound liposomes, other organelles, or Golgi condensing vesicles. Centrilobular glycogen was somewhat depleted in male rats receiving 1,500 mg/kg TCE. Periportal cells were ultrastructually normal in all rats.

For the morphometric analysis, the number of mice examined ranged from 7 in the control group to 8 in the 1,500 mg/kg TCE exposed group. The authors did not indicate which control animals were used for the morphometric analysis from the 75 animals examined for mitotic index, the 20 examined by light microscopy, or the 30 mice used as concurrent controls in the liver weight, DNA concentration, and tritiated thymidine incorporation studies. The authors stated that morphometry was performed on three randomly selected photomicrographs from each of three randomly selected pericentral hepatocytes for each animal (i.e., nine photomicrographs per animal). A mean value representing the exposure group was reported with the variability between photomitographs per animal or the variation between animals unclear. The morphometric analysis did not examine all treatment groups (e.g., only the control and 500 mg/kg TCE group were examined in Alderly Park mice). The percent cytoplasmic volume of the peroxisomal compartment (mean ± standard deviation [SD]) was reported to be 0.6% ± 0.6% for controls, 4.8% ± 3.3% for 500 mg/kg TCE, 6.7% ± 1.9% for 1,000 mg/kg TCE, and 6.4% ± 2.5% for 1,500 mg/kg TCE in B6C3F1 mice. In Alderly Park mice, only 12 control and 12 500 mg/kg TCE exposed mice were examined and, similarly, their selection criteria was not given. The percent cytoplasmic volume of the peroxisomal compartment was 1.2% ± 0.4% for control and 4.7 ± 2.8% for 500 mg/kg TCE exposed mice. For Osborne-Mendel rats control rats were reported to have a percent cytoplasmic volume of the peroxisomal compartment for control rats ($n = 9$) of 1.8% ± 0.4%, 1,000 mg/kg TCE ($n = 5$) 2.3% ± 1.6%, and for 1,500 mg/kg exposed rats ($n = 7$) 2.3% ± 2.0%. For Alderly Park rats only two groups were examined (control and 1,000 mg/kg TCE exposure). The percent cytoplasmic volume of the peroxisomal compartment for control rats ($n = 15$) was reported to be 1.8% ± 0.8% and for 1,000 mg/kg TCE ($n = 16$) to be 2.4% ± 1.2%. The varying numbers of animals examined, the varying and inconsistent number of treatment groups examined, the limited number of photomitographs per animal, and the potential selection bias for animals examined make quantitative conclusions regarding this analysis difficult. Although control levels differed by a factor of 2 between the two strains of mice examined, as well as the number of control animals examined (7 vs. 12), it appears that the 500-mg/kg TCE-exposed B6C3F1 and Alderly Park mice had similar percentages of peroxisomal compartment in the pericentral cells examined (~4.8%). There also appeared to be little difference between 1,000 mg/kg TCE treated Osborne-Mendel and Alderly Park rats for this parameter (~2.4%). Although few animals were examined, there was little difference reported between 500, 1,000, and 1,500 mg/kg TCE exposure groups in regard to percentages of peroxisomal compartment in B6C3F1 mice (4.8−6.7%). For the few rats of the Osborne-Mendel strain examined, there also did not appear to be a difference between 1,000 and 1,500 mg/kg TCE exposure for this parameter (2.3%).

Based on peroxisome compartment volume data, one would expect there to be little difference between TCE exposure groups in mice or rats in regard to enzyme activity or other "associated events." However, such comparisons are difficult due to limited power to detect differences and the possibility of bias in selection of animals in differing assays. For the B6C3F1 mice, only 5 animals per group were examined for enzyme analysis, 7 to 8 for morphometric analysis, 75 animals in control, and 20 animals in 1,000 mg/kg TCE-exposed groups for mitotic figure identification, and 10 animals per group for thymidine incorporation. Since only a few animals were tested for enzyme activity the comparison between peroxisomal compartment volume and that parameter is very limited. There was a reported 47% increase in catalase activity between control (n = 5) and 1,000 mg/kg TCE exposed B6C3F1 mice (n = 5) and 7.8-fold increase in PCO activity. The percent peroxisome compartment was reported to be 10.6-fold greater (0.6 vs. 6.4%). However, the B6C3F1 control percent volume of peroxisomal compartment was reported to be half that of the AP mouse control. An accurate determination of the quantitative differences in peroxisomal proliferation would be dependent on an accurate and stable control value. For Alderly Park rats there was an 8% decrease in catalase activity between control (n = 5) and 1,000 mg/kg TCE exposed rats (n = 5), and a 13% increase in PCO activity. The percent peroxisome compartment was reported to be 33% greater in the TCE-exposed than control group. Thus, for the very limited data that was available to compare peroxisomal compartment volume with enzyme activity, there was consistency in result.

However, were such increases in peroxisomes associated with other events reported in this study? Mouse peroxisome proliferation associated enzyme activities in B6C3F1 mice at 1,000 mg/kg TCE were reported to be 8-fold over control values in mice after 10 days of treatment. However, this increase in activity was not accompanied by a similar increase in thymidine incorporation (2.8-fold of control) or concordant with increases in mitotic figures (7/20 mice having any mitotic figures at all with a range of 0−5 and a mean of 0.014% of cells undergoing mitosis for 1,000 mg/kg TCE vs. 0 for control). Although results reported in the rat showed discordance between thymidine incorporation and detection of mitotic figures, there was also discordance with these indices and those for peroxisomal proliferation. In comparison to controls, there was a reported 13% increase in PCO activity in Alderly park rats exposed to 1,000 mg/kg TCE, a group mean of mitotic figures half that in the TCE treated animals versus controls, and increase in thymidine incorporation of 40%. Thus, these results are not consistent with TCE induction of peroxisome enzyme activity to be correlated with hepatocellular proliferation by either mitotic index or thymidine incorporation. Thymidine incorporation in liver DNA seen with TCE exposure also did not correlate with mitotic index activity in hepatocytes and suggests that this parameter may be a reflection of polyploidization rather than hepatocyte proliferation. More importantly, these data show that hepatocyte proliferation, indicated by either measure, is confined to a very small population of cells in the liver after 10 days of TCE exposure. Hepatocellular hypertrophy in the centrilobular region appears to be responsible for the liver weight gains seen in both rats and mice rather than increases in cell number. These results at 10 days do not preclude the possibility that a greater level of hepatocyte proliferation did not occur earlier and then had subsided by 10 days, as is characteristic of many mitogens. Thymidine incorporation represents the status of the liver at one time point rather than over a period of whole week and thus, would not capture the earlier bouts of proliferation. However, there is no evidence of a sustained proliferative response, as measured at the 10-day time period, in hepatocytes in response to TCE indicated from these data.

In regards to weight gain, although the volume of the peroxisomal compartment was reported to be similar at 500 mg/kg TCE in B6C3F1 and Alderly Park mice (4.3%), the liver weight./body weight gain in comparison to control was 20% higher in B6C3F1 mice versus 43% higher in Alderly Park mice after 10 days of exposure. The liver/body weight ratio was 5.53% in the B6C3F1 mice and 7.31% in the Alderly Park mice at 500 mg/kg TCE for 10 days. Similarly, although the peroxisomal compartment was similar at 1,000 mg/kg TCE in Osborne-Mendel (2.3%) and Alderly Park rats (2.4%), the liver weight/body weight gain was 26% in Osborne-Mendel rats but 17% in Alderly Park rats at this level of TCE exposure. The liver/body weight ratio was 5.35% in the Osborne-Mendel rats and 5.83% in the Alderly Park mice at 1,000 mg/kg TCE for 10 days. Although there are several limitations regarding the quantitative interpretation of the data, as discussed above, the data suggest that liver weight and weight gain after TCE treatment was not just a function of peroxisome proliferation. This study does clearly demonstrate TCE-induced changes at the lowest level tested in several parameters without toxicity and without evidence of regenerative hyperplasia or sustained hepatocellular proliferation. In regards to susceptibility to liver cancer induction in more susceptible (B6C3F1) versus less susceptible (Alderly Park/Swiss) strains of mice (Maltoni et al., 1988), there was a greater baseline level of liver weight/body weight ratio change, a greater baseline level of thymidine incorporation as well as greater responses for those endpoints due to TCE exposure in the "less susceptible" strain. However, both strains showed a hepatocarcinogenic response to TCE induction and the limitations of being able to make quantitative conclusions regarding species and strain susceptibility TCE toxicity from this study have been described in detail above.

E.2.1.9. *Dees and Travis, 1993*

The focus of this study was to evaluate the nature of DNA synthesis induced by TCE exposure in mice. The mitotic rate of liver cells was extrapolated using tritiated thymidine uptake into DNA of male and female mice treated with HPLC grade (99 + pure) TCE. Male and female hybrid B6C3F1 mice 8 weeks of age (male mice weighed 24−27 g (~12% difference) and females weighing 18−21 g (~4% difference) were dosed orally by gavage for 10 days with 100, 250, 500, and 1,000 mg/kg body weight TCE in corn oil (n = 4 per treatment group). 16 hours after the last daily dose of TCE, mice received tritiated thymidine and were sacrificed 6 hours later. Hepatic DNA was extracted form whole liver and standard histopathology was also performed. Hepatic DNA content and cellular distributions were also determined for thymidine uptake using autoradiography of tissue sections. Tritiated thymidine incorporation into DNA was determined by microscopic observations of autoradiography slides and reported as positive cells per 100 (200× power) fields.

Changes in the treatment groups were reported to

> include an increase in eosinophilic cytoplasmic staining of hepatocytes located near central veins, accompanied by loss of cytoplasmic vacuolization. Intermediate zones appeared normal and no changes were noted in portal triad areas. Male and female mice given 1,000 mg/kg body weight TCE exhibited apoptosis located near central veins. No evidence of cellular proliferation was seen in the portal areas. No evidence of increased lipofuscin was seen in liver sections from male and female mice treated with TCE. Evaluation of cell death in male and female mice receiving TCE was performed by enumerating apoptoses.

The apoptoses "did not appear to be in proportion to the applied TCE dose given to male or female mice." The mean number of apoptosis per 100 (400×) fields in each group of 4 animals (male mice) was 0, 0, 0, 1, and 8 for control, 100, 250, 500, and 1,000 mg/kg TCE treated groups, respectively. Variations in number of apoptoses between mice were not given by the authors. Feulgen stain was <1 for all doses except for 9 at 1,000 mg/kg.

Mitotic figure were reported to be

> frequently seen in liver sections from both male and female mice treated with TCE. Dividing cells were most often found in the intermediate zone and resembled mature hepatocytes. Incorporation of the radiolabel into cells located near the portal triad areas was rare. In general, mitotic figures were very rare, but when found they were usually located in the intermediate zone. Little or no incorporation of label was seen in areas near the bile duct epithelia or in areas close to the portal triad.

No quantitative description of mitotic index was reported by the authors but this description is consistent with there being replication of mature hepatocytes induced by TCE.

The distribution of tritiated thymidine was given for specific cell types in the livers of 5 animals per treatment group and radiolabel was reported to be predominantly associated with perisinusoidal cell in control mice. The authors state that the label was more often found in cells resembling mature hepatocytes. The mean number of labeled cells in autoradiographs per 100 (200× power) fields was reported to be ~125 and ~150 labeled perisinusoidal cells in controls male and female mice, respectively. The authors do not give any standard deviations for the female perisinusoidal data except for the 1,000-mg/kg exposure group. For mature hepatocytes, the mean baseline level of cell labeling for control male and female mice were reported to be ~65 and ~90 labeled cells, respectively. Although the baseline levels of hepatocyte labeling were reported to differ between male and female mice, the mean peak level of labeling was similar at ~250 labeled cells for male and female mice treated with TCE. Thus, in male mouse liver, the number of labeled cells increased ~2-fold of control levels after 500 and 1,000 mg/kg TCE and in female mouse liver increased ~4-fold of control levels after 250, 500, and 1,000 mg/kg TCE in female mouse liver hepatocytes over their respective control levels.

Incorporation of tritiated thymidine into DNA extracted from whole liver in male and female mice was reported to be significantly elevated after TCE treatment but, unlike the autoradiographic data, there was no difference between genders and the mean peak level of tritiated thymidine incorporation occurred at 250 mg/kg TCE treatment and remained constant for the 500 and 1,000 mg/kg treated groups. Increased thymidine incorporation into DNA extracted from liver of male and female mice were reported to show a very large standard deviation with TCE treatment (e.g., at 100 mg/kg TCE exposure, male mice had a mean of ~130 dpm tritiated thymidine/microgram DNA with the upper bound of the standard deviation to be 225 dpm). The increased thymidine incorporation peaked at a level that was a little less than 2-fold of control level. Thus, for both male and female mice both autoradiographs and total hepatic DNA were reported to show that male and female mice had similar peaks of increased thymidine incorporation after TCE exposure that reached a plateau at the 250 mg/kg TCE exposure level and did not increase with increasing exposure concentration. These data also indicate a very small population undergoing mitosis due to TCE exposure after 10 days of exposure. If higher levels of hepatocyte replication had occurred earlier, such levels were not sustained by 10 days of TCE exposure. More importantly, these data suggest that tritiated thymidine levels were targeted to mature hepatocytes and in areas of the liver where greater levels of polyploidization. The ages and weights of the mice were described by these authors, unlike Elcombe et al, and a different strain was used. However, these results are consistent with those of Elcombe in regard to the magnitude of thymidine incorporation induced by TCE treatment and the lack of a dose response once a relative low level of exposure has been exceeded.

The total liver DNA content of male and female mice treated with TCE were also determined with the total micrograms DNA/g liver reported to be ~4 microgram/g for female control mice and ~2 micrograms/g for male control mice. Although not statistically significant, the total DNA concentration dropped from ~4 to ~3 at 100 mg/kg through 1,000 mg/kg exposure to TCE in female mice. For male mice the total DNA rose slightly in the 250- and 500-mg/kg groups to ~3 micrograms/gram and was similar to control levels at the 100 and 1,000 mg/kg TCE treatment groups. The standard deviation in male mice was very large and the number of animals small making quantitative judgments regarding this parameter difficult. The slight decrease reported for female mice would be consistent with the results of Elcombe et al. (1985) who describe a slight decrease in hepatic DNA in male mice. However, the reported slight increase in hepatic DNA in male mice in this study is not consistent. Given the small number of animals and the large deviations for female and male mice in the TCE treated groups, this study may not have had the sensitivity to detect slight decreases reported by Elcombe et al.

In regard to clinical evaluation and weight analyses, both male and female mice given TCE were reported "to appear clinically ill. These mice showed reduced activity and failed to groom. Control mice showed no adverse effects. Female mice were markedly more affected by TCE than their male counterparts. Several deaths of female mice occurred during the course of the TCE treatment regimen." The authors do not give cause of deaths but state that two female mice died in the group receiving 250 mg/kg TCE and one in the group receiving 1,000 mg/kg during the gavage regimen of the female mice. This appears to be similar gavage error or "accidental death" reported in National Toxicology Program (NTP) studies chronic studies of TCE (see below).

The authors report

> no significant difference in the absolute body weight of male and female mice were noted in control groups. Body weight gain in female and males mice treated with TCE was not significantly different from that of control mice. Liver weights in male mice given 500 or 1,000 mg/kg and corrected for total body weight were significantly elevated. The corrected liver weights of female mice increase proportionally with the applied dose of TCE.

For male mice, liver weights were reported to be 1.40 ± 0.16, 1.38 ± 1.23, 1.48 ± 0.09, 1.61 ± 0.07, and 1.63 ± 0.11 g for control, 100, 250, 500, and 1,000 mg/kg TCE in male mice (*n* = 5), respectively. Body weights were smaller for the 100 mg/kg TCE treatment group although not statistically significant. The liver weights after treatment had a much larger reported standard deviation (1.23 g for 100 mg/kg group vs. <0.16 for all other groups). The percent liver/body weight ratios were reported to be 5.40, 5.41, 5.42, 5.71, and 6.34% for the same groups in male mice. This represents 1.06- and 1.17-fold of control at the 500 and 1,000 mg/kg dose. The authors report a statistically significant increase in percent liver/body weight ratio only for the 500 mg/kg (i.e., 1.06-fold of control) and 1,000 mg/kg (i.e., 1.17-fold of control) TCE exposure groups. The results for female mice liver weights were reported in Table III of the paper, which was mistakenly labeled as for male mice. The reported values for liver weight were 1.03 ± 0.07, 1.05 ± 0.10, 1.15 ± 0.98, 1.21 ± 0.18, and 1.34 ± 0.08 g for control, 100, 250, 500, and 1,000 mg/kg TCE in female mice (*n* = 5, except for 250 mg/kg and 1,000 mg/kg groups), respectively. The percent liver/body weight ratios were 5.26, 5.44, 5.68, 6.24, and 6.57% for the same groups. These values represent 1.03-, 1.08-, 1.19-, and 1.25-fold of controls in percent liver/body weight. The magnitude of increase in TCE-induced percent liver/body weight ratio in female mice is reflective of the magnitude of the difference in dose up to 1,000 mg/kg where it is slightly lower. The female mice were reported to have statistically significant increases in percent liver/body ratios at the lowest dose tested (100 mg/kg TCE) after 10 days of TCE exposure that also increased proportionately with dose. Male mice were not reported to have a significant increase in percent liver/body weight until 500 mg/kg TCE but a statistically significant increase in liver weight at 250 mg/kg TCE. Male mice had a much larger variation in initial body weight than did female mice (range of means of 24.86 to 27.84 g between groups for males or ~11% difference and range of means of 19.48 to 20.27 g for females or ~4%) which may contribute to an apparent lack of effect for a parameter that is dependent on body weight. Only 5 mice were used in each group so the power to detect a change was relatively small.

The results from this experiment are consistent with those of Elcombe et al. (1985) in showing a slight increase in thymidine incorporation (~2-fold of control) and mitotic figures that are rare after TCE exposure. This study also records a lack of apoptosis with TCE treatment except at the highest exposure level (i.e., 1,000 mg/kg). The increases in liver weight induced by TCE were reported to be dose-related, especially in female mice where baseline body weights were more consistent. However, the incorporation of tritiated thymidine reached a plateau at 250 mg/kg TCE in the DNA of both genders of mice. This study specifically identified where thymidine incorporation and mitotic figures were occurring in TCE-treated livers and noted that the mature hepatocyte that appeared to be primarily affected, as well as in the portion of the liver where mature hepatocytes with higher ploidy are found. The authors note that the "lack of thymidine incorporation in the periportal area, where the liver stem cells are reside," suggesting that the mature hepatocyte is the target of TCE effects on DNA synthesis. This finding is consistent with a change in ploidy accompanying hepatocellular hypertrophy and not just cell proliferation after 10 days of TCE exposure. Like Elcombe et al. (1985), these data represent "a snapshot in time" which does not show whether increased cell proliferation may have happened at an earlier time point and then subsided by 10 days. However, like Elcombe et al. (1985) it suggests that sustained proliferation is not a feature of TCE exposure and that the level of DNA synthesis (which is very low in quiescent control liver) is increased in a small population of hepatocytes due to TCE exposure that is not dose-dependent (only 2-fold increase over control in animals exposed from 250 to 1,000 mg/kg TCE). In regards to toxicity, no evidence of increased lipid peroxidation in TCE-treated animals was reported using histopathologic sections stained to enhance observation of lipofuscin. No necrosis is noted by these authors and the deaths in female mice are likely due to gavage error.

E.2.1.10. *Nakajima et al., 2000*

This study focused on the effect of TCE treatment on PPARα-null mice in terms of peroxisome proliferation but also included information on differences in liver weight between null and wild-type mice, as well as gender-related effects. SV129 wild-type and PPARα-null mice (10 weeks of age) were treated with corn oil or 750 mg/kg TCE in corn oil daily for 2 weeks via gavage (n = 6 per group). A small portion of the liver was removed for histopathological examination but the lobe used was not specified by the authors. Liver peroxisome proliferation was reported to be evaluated morphologically using 3,3'-diaminobenzidine (DAB) staining of sections and electron photomicroscopy to detect the volume density of peroxisomes (percent of cytoplasm) in 15 micrographs of the pericentral area per liver. A number of β-oxidation enzymes and P450s were analyzed by immunoblot of liver homogenates.

The final body weights, liver weights and percent liver/body weight ratios were reported for all treatment groups. For male mice, vehicle treated PPARα-null mice had slightly lower mean body weights (24.5 ± 1.8 g vs. 25.4 ± 1.9 g [SD]), slightly larger liver weights (1.14 ± 0.13 g vs. 1.05 ± 0.15 g or ~9%), and slightly higher percent liver/body weight ratios (4.12% ± 0.32% vs. 4.10% ± 0.37%) than wild-type mice. The mean values for final body weights of the groups of mice in this study were reported and were similar which, as demonstrated by the inhalation studies by Kjellstrand et al. (1983a) (see Section E.2.2.4), is particularly important for determining the effects of TCE treatment on percent liver/body weight ratios. For both groups of male mice, 2 weeks of TCE treatment significantly increased both liver weight and percent liver/body weight ratios. For male wild-type mice the increase in percent liver/body weight was 1.50-fold of vehicle control and for male PPARα-null mice the increase was 1.26-fold of control after 2 weeks of TCE treatment. For female mice, vehicle treated PPARα-null mice had slightly higher mean body weights (22.7 ± 2.1 g vs. 22.4 ± 2.0 g), slightly larger liver weights (0.98 ± 0.15 g vs. 0.95 ± 0.14 g or ~3%), and slightly higher percent liver/body weight ratios (4.32% ± 0.35% vs. 4.24% ± 0.41%) than wild-type mice. For both groups of female mice, 2 weeks of TCE treatment significantly increased percent liver/body weight ratios. For liver weights there was a reporting error for PPARα-null female treated with TCE so that liver weight changes due to TCE treatment cannot be determined for this group. For female wild-type mice the increase in percent liver/body weight was 1.24-fold of vehicle control and for female PPARα-null mice the increase was 1.26-fold of control after 2 weeks of TCE treatment. Thus, for both wild-type and PPARα-null mice, TCE exposure resulted in increased percent liver/body weight over controls that was statistically significant after 2 weeks of oral gavage exposure using corn oil as the vehicle. For male mice there was a greater TCE-induced increase in percent liver/body weight in wild-type than PPARα-null mice (1.50- vs. 1.26-fold of control) that was statistically significant, but for female mice the induction of increased liver weight was statistically increased but the same in wild-type and PPARα-null mice (i.e., both were ~1.25-fold of control). These date indicate that TCE-induced increases in mouse liver weight were not dependent on a functional PPARα receptor in female mice and suggest that some portion may be in male mice.

In regard to light and electron microscopic results, the numbers of peroxisomes in hepatocytes of wild-type mice were reported to be increased, especially in the pericentral area of the hepatic lobule, to a similar extent in both males and females (15 micrographs, $n = 4$ mice). TCE exposure was reported to increase the volume density of peroxisomes 2-fold of control in the pericentral area with no evident change in peroxisomes in the periportal areas, but data was not shown for that area of the liver lobule. In contrast, no increase in peroxisomes was reported to be observed in PPARα-null mice. Therefore, increases in liver weight observed in PPARα-null mice after TCE treatment did not result from peroxisome proliferation. Similarly, the small 2-fold increase in peroxisome volume from 2 to 4% of cytoplasmic volume in the pericentral area of the liver lobule in wild-type mice could not have been responsible for the 50% increase liver weight observed in male wild-type mice.

Although no difference was reported between male and female wild-type mice in regard to TCE-induced peroxisome proliferation in wild-type mice, the levels of hepatic enzymes associated with peroxisomes (acyl-CoA [AOX], peroxisomal bifunctional protein [PH], peroxisomal thiolase [PT], very long chain acyl-CoA synthetase, and D-type peroxisomal bifunctional protein [DBF], cytosolic enzyme [cytosolic thioesterase II (CTEII)], mitochondrial enzymes [mitochondrial trifunctional protein α subunits α and β(TPα and TPβ)], and microsomal enzymes [cytochrome P450 4A1 (CYP4A1)]) as measured by immunoblot analysis were significantly elevated in male wild-type mice ($n = 4$) by a factor of ~2−3, but except for a slight elevation in PH and PT, were reported to not be elevated in female wild-type mice ($n = 4$). The magnitude of increase in peroxisomal enzymes was similar to that of peroxisomal volume in male mice. No TCE-induced increases in any of these enzymes were reported in male or female PPARα-null mice by the authors. For CYP4A1, an enzyme reported to be induced by peroxisomal proliferators, TCE exposure resulted in a much lower amount in female than male wild-type mice (i.e., 2% of the level induced by TCE in males). However, the expression of catalase was reported to be "nearly constant in all samples" (at most ~30% change) which the authors suggested resulted from induction by TCE that was independent of PPARα. The basis for selection of 4 mice for this comparison out of the 6 studied per group was not given by the authors. A comparison of control wild-type and PPARα-null mice showed that in males background levels of the enzymes examined were generally similar except for DBF in which the null mice had values ~50% of the wild-type controls. A similar decrease was reported for female PPARα-null mice. With regard to gender differences in wild-type mice, females had similar values as males with the exceptions of TPα, TPβ, and CYP2E1 which were in untreated female wild-type mice at a 3.06-, 2.38-, and 1.63-fold for l TPα, TPβ, and CYP2E1 levels over males, respectively. Female PPARα-null mice had increases of 2.50-, 1.54-, and 2.07-fold over male wild-type mice.

With regard to the induction of TCE metabolizing enzymes (CYP1A2, CYP2E1, and ALDH), CYP1A2 was reported to be decreased by TCE treatment of both male and female wild-type mice but liver CYP2E1 reported to be increased in male mice and constant in female mice which resulted in similar expression level in both genders after TCE treatment. There was no gender difference in ALDH activity reported after TCE exposure and activity was reported to be independent of PPARα. The authors concluded that TCE metabolizing abilities of the liver of male and female mice were similar and therefore, poor induction of peroxisomal related enzymes was not due to gender-related differences in TCE metabolism.

To investigate whether the a gender-related difference peroxisomal enzymes after TCE exposure was due to a lower levels of PPARα and RXRα receptors, western blotting was employed ($n = 3$). The level of PPARα protein was reported to be increased in both male wild-type mice with less induction in females (control vs. TCE, 1.00 ± 0.20 vs. 2.17 ± 0.24 in males and 0.95 ± 0.25 vs. 1.44 ± 0.09 in females) after TCE treatment. The hepatic level of RXRα was also reported to be increased in the same manner as PPARα (control vs. TCE, 1.00 ± 0.33 vs. 1.92 ± 0.04 in males 0.81 ± 0.16 vs. 1.14 ± 0.10 in females). Northern blot analysis of hepatic PPARα mRNA was reported to show greater TCE induction in male (2.6-fold of control) than in female (1.5-fold of control) wild-type mice. Thus, males appeared to have higher induction of the two receptor proteins as well as a greater response in peroxisomal enzymes and CYP4A1, even though TCE-induced increases in peroxisomal volume was similar between male and female mice. The increased response in males for induction of the two receptor proteins is consistent with liver weight data that shows some portion of the induction of increased liver weight response in male mice using this paradigm may be due to gender-specific differences in PPARα response. However, as noted below (see Section E.2.2), corn oil vehicle has liver effects alone, especially in the male liver, that have also been associated with PPARα responses.

E.2.1.11. *Berman et al., 1995*

This study included TCE in a suite of compounds used to compare endpoints for toxicological screening methods. Female Fischer 344 rats of 77 days of age ($n = 8$ per group) were administered TCE in corn oil for 1 day (0, 150, 500, 1,500, or 5,000 mg/kg/d) or for 14 days (0, 50, 150, 500, or 1,500 mg/kg/d). Blood samples were taken 24 hours after the last dose and livers were weighed and H&E sections were examined for evidence of parenchymal cell degeneration, necrosis, or hypertrophy. No details were provided by the authors for the extent or severity of the liver affects by histopathological examination. The serum chemistry analysis included lactate dehydrogenase (LDH), alkaline phosphatase, ALT, aspartate aminotrasferase (AST), total bilirubin, creatine, and blood urea nitrogen. The starting and ending body weights of the animals or the absolute liver weights were not reported by the authors.

The results of a multivariate analysis were reported to show a lowest effective dose of 1,500 mg/kg after 1 day of TCE exposure and 150 mg/kg after 14 days of TCE exposure that was statistically significant. Liver weight and liver weight changes were not reported by the authors but the percent liver to body weight ratios were. For the two control groups there was a difference in percent liver/body weight of ~8% (3.43% ± 0.74% for the 1-day control group and 3.16% ± 0.41% for the 14-day control group, mean ± SEM). For the 1-day groups only the 5,000 mg/kg group was reported to show a statistically significant difference in percent liver/body weight between control and TCE treatment (i.e., ~1.08-fold increase). Hepatocellular necrosis was noted to occur in the 1,500 and 5,000 mg/kg groups in 6/7 and 6/8 female rats, respectively but not to occur in lower doses. The extent of necrosis was not noted by the authors for the two groups exhibiting a response after 1 day of exposure. Serum enzymes indicative of liver necrosis were not presented and because only positive results were presented in the paper, presumed to be negative. Therefore, the extent of necrosis was not of a magnitude to affect serum enzyme markers of cellular leakage.

After 14 days of TCE exposure, there was a dose-related increase reported for percent liver/body weight ratios that was statistically significant at all TCE dose levels although the multivariate analysis indicated the lowest effective dose to be 150 mg/kg. The percent liver/body weight ratio was 3.16% ± 0.41%, 3.38% ± 0.56%, 3.49% ± 0.69%, 3.82% ± 0.76%, and 4.47% ± 0.66% for control, 50, 150, 500, and 1,500 mg/kg TCE exposure levels, respectively after 14 days of exposure. No hepatocellular necrosis was reported at any dose and hepatocellular hypertrophy was reported only at the 1,500 mg/kg dose and in all rats. These rat liver weights are 1.07-, 1.10-, 1.21-, and 1.41-fold of controls for the 50, 150, 500, and 1,500 mg/kg TCE dose groups, respectively. The 7% increase in liver weight at the 50 mg/kg dose is approximately the same difference between the two control groups for Days 1 and 14 treatments. Without the data for starting and final body weights and an examination of whether the control animals had similar body weight, it is impossible to discern whether the reported effects at the low dose of TCE was also reflected differences between the control groups. No serum enzyme levels changes were reported after 14 days of exposure to TCE for any group.

The authors note that their study provided evidence of liver effects at lower levels than other studies citing Elcombe et al. (1985) and Goldsworthy and Popp (1987). They suggest that the differences in sensitivity to TCE between their results and those of these two studies may reflect differences in strain or gender of the rats examined. However, they did not study male rats of this strain concurrently so that differences in gender may have reflected differences between experiments. The increase in liver weight without reporting increases in hepatocellular hypertrophy as well as the lack of necrosis as low doses is consistent with the results of Melnick et al. (1987) in male Fischer rats given TCE orally (see Section E.2.1.11, below).

E.2.1.12. *Melnick et al., 1987*

The focus of this study was to assess microencapsulation as a way to expose rodents to substances such as TCE that have issues related to volatilization in drinking water or apparent gavage-related deaths. In this study, liver weight changes, extent of focalized necrosis, and indicators of peroxisome proliferation were reported as metrics of TCE toxicity. TCE (99+ %) was encapsulated in gelatin-sorbitol microcapsules and was 44.1% TCE w/w. The TCE microcapsules were administered to male Fischer 344 rats (6-week old and weighing between 89 and 92 g or ~3% difference) in the diet (0, 0.55, 1.10, 2.21, and 4.42% TCE in the diet) for 14 days. The number of animals in each group was 10. A parallel group of animals was administered TCE in corn oil gavage for 14 consecutive days (corn oil control, 0.6, 1.2, and 2.8 g/kg/day TCE). The dosage levels of TCE in the gavage study were reported to be "adjusted 5 times during the 14-day" treatment period to be similar to the dosage levels of TCE in the feed study. The time-weighted average dosage levels of TCE in the feed study were reported to be 0.6, 1.3, 2.2, and 4.8 g/kg/day.

There was less food consumption reported in the 2.2 and 4.8 g/kg/day dose feed groups, which the authors attribute to either palatability or toxicity. There were no deaths in any of the groups treated with microencapsulated TCE while, similar to many other gavage studies of TCE reported in the literature, there were 4 deaths in the high-dose gavage group. Mean body weight gains of the two highest dose groups of the feed study and of the highest dose group of the gavage study were reported to be significantly lower than the mean body weight gains of the respective control groups (i.e., ~22 and ~35% reduction at 2.2 and 4.8 g/kg/day in the feed study, respectively, and ~33% reduction at 2.8 g/kg/day TCE in the gavage study). After 14 days of treatment, liver weights were reported to be 8.1 ± 0.8, 8.4 ± 0.8, 9.5 ± 0.5, 10.1 ± 1.2, 8.9 ± 1.3, and 7.4 ± 0.5 g for untreated control, placebo control, 0.6, 1.3, 2.2, and 4.8 g/kg TCE exposed feed groups, respectively. The corresponding percent liver/body weight ratios were reported to be 5.2% ± 0.3%, 5.3% ± 0.2%, 6.0% ± 0.3%, 6.5% ± 0.5%, 7.0% ± 0.9%, and 7.1% ± 0.5% for untreated control, placebo control, 0.6, 1.3, 2.2, and 4.8 g/kg TCE exposed groups, respectively. The increased percent liver/body weight ratio represents 1.13-, 1.23-, 1.32-, and 1.34-fold of placebo controls, respectively. For the gavage experiment, after 14 days of treatment liver weights were reported to be 7.1 ± 1.3, 9.3 ± 1.2, 9.1 ± 0.9, and 7.7 ± 0.4 g for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. The corresponding percent liver/body weight ratios were reported to be 5.0% ± 0.4%, 6.0% ± 0.4%, 6.1% ± 0.3%, and 7.3% ± 0.5% for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. The percent liver/body weight ratios represent 1.20-, 1.22-, and 1.46-fold of corn oil controls, respectively. The 2.8 g/kg TCE gavage results are reflective of the 6 surviving animals in the group rather than 10 animals in the rest of the groups. There was no explanation given by the authors for the lower liver weights in the control gavage group than the placebo control in the feed group (i.e., 20% difference) although the initial and final body weights appeared to be similar. The decreased body weights in the feed and gavage study are reflective if TCE systemic toxicity and appeared to affect the TCE-induced liver weight increases in those groups.

The authors reported that the only treatment-related lesion observed microscopically in rats from either dosed-feed or gavage groups was individual cell necrosis of the liver with the frequency and severity of this lesion similar at each dosage levels of TCE administered microencapsulated in the feed or in corn oil. Using a scale of minimal = 1−3 necrotic hepatocytes/10 microscopic 200× fields, mild = 4−7 necrotic necrotic hepatocytes/10 microscopic 200× fields, and moderate = 8−12 necrotic hepatocytes/10 microscopic 200× fields, the frequency of lesion was 0−1/10 for controls, 2/10 for 0.6 and 1.3 g/kg and 9/10 for 2.2 and 4.8 g/kg feed groups. The mean severity was reported to be 0.0−0.1 for controls, 0.3−0.4 for 0.6 and 1.3 g/kg, and 2.0−2.5 for 2.2 and 4.8 g/kg feed groups. For the corn oil gavage study, the corn oil control and 0.6 g/kg groups were reported to have a frequency of 0 lesions/10 animals, the 1.2 g/kg group a frequency of 1/10 animals, while the 2.8 g/kg group to have a frequency of 5/6 animals. The mean severity score was reported to be 0 for the control and 0.6 g/kg groups, 0.1 for the 1.2 g/kg groups, and 1.8 for the remaining 6 animals in the 2.8 g/kg group. The individual cell necrosis was reported to be randomly distributed throughout the liver lobule with the change to not be accompanied by an inflammatory response. The authors also report that there was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells. Thus, although there appeared to be TCE-treatment related increases in focal necrosis after 14 days of exposure, the extent was even at the highest doses mild and involved few hepatocytes.

Microsomal NADPH cytochrome c-reductase was reported to be elevated in the 2.2 and 4.8 g/kg feed groups and in the 1.2 and 2.8 g/kg gavage groups. Cytochrome P450 levels were reported to be elevated only in the two highest dose groups of the feed study. The authors reported a dose-related increase in peroxisome PCO and catalase activities in liver homogenates from rats treated with TCE microcapsules or by gavage and that treatment with corn oil alone, but not placebo capsules, caused a slight increase in PCO activity. After 14 days of treatment, PCO activities were reported to be 270 ± 12, 242 ± 17, 298 ± 64, 424 ± 55, 651 ± 148, and 999 ± 266 nmol H_20_2 produced/min/g liver for untreated control, placebo control, 0.6, 1.3, 2.2, and 4.8 g/kg TCE exposed feed groups, respectively. This represents 1.23-, 1.75-, 2.69-, and

4.13-fold of placebo controls, respectively. After 14 days of treatment, catalase activities were reported to be 8.49 ± 0.81, 7.98 ± 1.62, 8.49 ± 1.92, 8.59 ± 1.31, 13.03 ± 2.01, and 15.76 ± 1.11 nmol H_20_2 produced/min/g liver for untreated control, placebo control, 0.6, 1.3, 2.2, and 4.8 g/kg TCE exposed groups, respectively. This represents 1.06-, 1.07-, 1.63-, and 1.97-fold of placebo controls, respectively. Thus, although reported to be dose related, only the two highest exposure levels of TCE increased catalase activity and to a smaller extent than PCO activity in microencapsulated TCE fed rats. For the gavage experiment, after 14 days of treatment PCO activities were reported to be 318 ± 27, 369 ± 26, 413 ± 40, and 1,002 ± 271 nmol hydrogen peroxide (H_2O_2) produced/min/g liver for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. This represents 1.16-, 1.29-, and 3.15-fold of corn oil controls. After 14 days of treatment, catalase activities were reported to be 8.59 ± 0.91, 10.10 ± 1.82, 12.83 ± 3.43, and 13.54 ± 2.32 nmol H_2O_2 produced/min/g liver for corn oil control, 0.6, 1.2, and 2.8 g/kg TCE exposed groups, respectively. This represents 1.18-, 1.49-, and 1.58-fold of corn oil controls. As stated by the authors the corn oil vehicle appeared to elevate catalase activities and PCO activities.

In regard to dose-response, liver and body weight were affected by decreased body weight gain in the higher dosed animals in this experiment (i.e., 2.2 g/kg/day TCE exposure and above) and by gavage related deaths in the highest-dosed group. The lower liver weight in the gavage control group also may have affected the determination of the magnitude of TCE-related liver weight gain at that dose. At the 2 doses, below which body weight gain was affected, there appeared to be an approximately 20% increase in percent liver/body weight ratio in the gavage study and a 13 and 23% weight increase in the feed study. The extent of PCO activity appeared to increase more steeply with dose in the feed study than did liver weight gain (i.e., a 1.23-fold of liver/body weight ratio at 1.3 g/kg/day corresponded with a 1.75-fold PCO activity over control). At the two highest doses in the feed study, the increase in PCO activity was 2.69- and 4.13-fold of control but the increase in liver weight was not more than 34%. For the gavage study, there was also a steeper increase in PCO activity than liver weight gain. For catalase activity, the increase was slightly less than that of liver/body weight ratio percent for the two doses that did not decrease body weight gain in the feed study. In the gavage study, they were about the same. In regard to what the cause of liver weight gain was, the authors report that there was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells and do not describe indicators of hepatocellular proliferation or increased polyploidy. Accordingly, the cause of liver weight gain after TCE exposure in this paradigm is not readily apparent.

E.2.1.13. *Laughter et al., 2004*

Although the focus of the study was an exploration of potential MOAs for TCE effects through macroarray transcript profiling (see Section E.3.1.2 for discussions of limitations of this approach and especially the need for phenotypic anchoring, Section E.3.4.1.3 for use of PPARα knockout mice, and Section E.3.4.2.2 for discussion of genetic profiling data for TCE), information was reported regarding changes in the liver weight of PPARα-null mouse and their background strains. SV129 wild-type and PPARα-null male mice (9 ± 1.5 weeks of age) were treated with 3 daily doses of TCE in 0.1% methyl cellulose for either 3 days or 3 weeks (*n* = 4−5/group). Thus, this paradigm does not use corn oil, which has been noted to affect toxicity (see Section E.2.2 below), but is not comparable to other paradigms that administer the total dose in one daily gavage administration rather than to give the same cumulative dose but in 3 daily doses of lower concentration. The initial or final body weights of the mice were not reported. Thus, the effects of systemic toxicity from TCE exposure on body weight and the influence of differences in initial body weight on percent liver/body weight determinations cannot be made. For the 3-day study, mice were administered 1,500 mg/kg TCE or vehicle control. For the 3-week study, mice were administered 0, 10, 50, 125, 500, 1,000, or 1,500 mg/kg TCE 5 days a week except for 4 day/week on the last week of the experiment. In a separate study, mice were given TCA or dichloroacetic acid (DCA) at 0.25, 0.5, 1, or 2 g/L (pH ~7) in the drinking water for 7 days. For each animal a block of the left, anterior right, and median liver lobes was reported to be fixed in formalin with 5 sections stained for H&E and examined by light microscopy. The remaining liver samples were combined and used as homogenates for transcript arrays. In the 3-week study, bromodeoxyuridine (BrdU) was administered via miniosmotic pump on day one of Week 3 and sections of the liver assessed for BrdU incorporation in at least 1,000 cells per animal in 10−15 fields.

Although initial body weights, final body weights, and the liver weights were not reported, the percent liver/body ratios were. In the 3-day study, control wild-type and PPARα-null mice were reported to have similar percent liver/body weight ratios of ~4.5%. These animals were ~10 weeks of age upon sacrifice. However, at the end of the 3-week experiment the percent liver/body weight ratios were increased in the PPARα-null male mice and were 5.1%. There was also a slight difference in the percent liver/body weight ratios in the 1-week study (4.3% ± 0.4% vs. 4.6% ± 0.2% for wild-type and PPARα-null mice, respectively). These results are consistent with an increasing baseline of hepatic steatosis with age in the PPARα-null mice and increase in liver weight. In the 3-day study, the mean reported the percent liver/body ratio was 1.4-fold of the animals tested with TCE in comparison to the control level. In the PPARα-null mice, there was a 1.07-fold of control level reported by the authors to not be statistically significant. However, given the low number of animals tested (the authors give only that 4−5 animals were tested per group without identification as to which groups has 4 animals and which had 5), the ability of this study to discern a statistically significant difference is limited. In the 3-week study, wild-type mice exposed to various concentrations of TCE had percent liver/body weights that were within ~2% of control values except for the 1,000 mg/kg and 1,500 mg/kg groups that were ~1.18- and 1.30-fold of control levels, respectively. For the PPARα-null mice exposed to TCE for 3 weeks, the variability in percent liver/body weight was greater than that of the wild-type mice in most of the groups. The baseline level percent liver/body weight was 1.16-fold in the PPARα-null mice in comparison to wild-type mice. At the 1,500 mg/kg TCE exposure level percent liver/body weights were not recorded because of the death of the null mice at this level. The authors reported that at the 1,500 mg/kg level all PPARα-null mice were moribund and had to be removed from the study. However, at 1,000 mg/kg TCE exposure level there was a 1.10-fold of control percent liver/body weight value that was reported to not be statistically significant. However, as noted above, the power of the study was limited due to low numbers of animals and increased variability in the null mice groups. The percent liver/body weight reported in this study was actually greater in the null mice than the wild-type male mice at the 1,000 mg/kg TCE exposure level (5.6% ± 0.4% vs. 5.2% ± 0.5%, for null and wild-type mice, respectively). Thus, at 1-week and at 3-weeks, TCE appeared to induce increases in liver weight in PPARα-null mice, although not reaching statistical significance in this study, with concurrent background of increased liver weight reported in the knockout mice. At 1,000 mg/kg TCE exposure for 3 weeks, percent liver/body weight was reported to be 1.18-fold in wild-type and 1.10-fold in null mice of control values. As discussed above, Nakajima et al. (2000) reported statistically significant increased liver weight in both wild-type and PPARα-null mice after 2 weeks of exposure with less TCE-induced liver weight increases in the knockout mice (see Section E.2.1.10). They also used more mice, carefully matched to weights of their mice, and used a single dose of TCE each day with corn oil gavage.

The authors noted that inspection of the livers and kidneys of the moribund null mice, who were removed from the 3-week study, "did not reveal any overt signs of toxicity in this dose group that would lead to morbidity" but did not show the data and did not indicate when the animals were affected and removed. For the wild-type mice exposed to the same concentration (1,500 mg/kg) but whose survival was not affected by TCE exposure, the authors reported that at the 1,500 mg/kg dose these mice exhibited mild granuloma formation with calcification or mild hepatocyte degeneration but gave not other details or quantitative information as to the extent of the lesions or what parts of the liver lobule were affected. The authors noted that "wild-type mice administered 1000 and 1500 mg/kg exhibited centrilobular hypertrophy" and that "the mice in the other groups did not exhibit any gross pathological changes after TCE exposure." Thus, the hepatocellular hypertrophy reported in this study for TCE appeared to be correlated with increases in percent liver/body weight in wild-type mice. In regard to the PPARα-null mice, the authors stated that "differences in the liver to body weights in the control PPARα-null mice between Study 1 and 2 the 3-day and 3-week studies] were noted and may be due to differences in the degree of steatosis that commonly occurs in this strain." Further mention of the background pathology due to knockout of the PPARα was not discussed. The increased percent liver/body weight reported between control and 1,000 mg/kg TCE exposed mice (5.1 vs. 5.6%) was not accompanied by any discussion of pathological changes that could have accounted for the change.

Direct comparisons of the effects of TCE, DCA, and TCA cannot be made from this study as they were not studied for similar durations of exposure. However, while TCE induced increased in percent liver/body weight ratios after 3 days and 3 weeks of exposure in wild-type mice at the highest dose levels, for TCA exposure percent liver/body weight after 1 week exposure in drinking water was slightly elevated at all dose levels with no dose-response (~10% increase), and for DCA exposure in drinking water a similar elevation in percent liver/body weight was also reported for the 0.25, 0.5, and 1.0 g/L dose levels (~11%) and that was increased at the 2.0 g/L level by ~25% reaching statistical significance. The authors interpret these data to show no TCA-related changes in wild-type mice but the limited power of the study makes quantitative conclusions difficult. For PPARα-null mice all there was a slight decrease in percent liver/body weight between control and TCA treated mice at the doses tested (~2%). For DCA-treated mice, all treatment levels of DCA were reported to induce a higher percent liver/body weight ratio of at least ~5% with a 13% increase at the 2.0 g/L level. Again the limited power of the study and the lack of data for TCE at similar durations of exposure as those studied for TCA and DCA makes quantitative conclusions difficult and comparisons between the chemicals difficult. However, the pattern of increased percent liver/body weight appears to be more similar between TCE and DCA than TCA in both wild-type and PPARα-null mice. In terms of histological description of effects, the authors note that "livers from the 2 g/L DCA-treated wild-type and PPARα-null mice had hepatocyte cytoplasmic rarefication probably due to an increase in glycogen accumulation." However, no special procedures are staining were performed to validate the assumption in this experiment. No other pathological descriptions of the DCA treatment groups were provided. In regard to TCA, the authors noted that "the livers from wild-type but not PPARα-null mice exposed to 2.0g/L TCA exhibited centrilobular hepatocyte hypertrophy." No quantitative estimate of this effect was given and although the extent of increase of percent liver/body weight was similar for all dose levels of TCA, there is no indication from the study that lower concentrations of TCA also increased hepatocellular hypertrophy or why there was no concurrent increase in liver weight at the highest dose of TCA in which hepatocellular hypertrophy was reported. Thus, reports of hepatocellular hypertrophy for DCA and TCA in the 1-week study were not correlated with changes in percent liver/body weight.

For control animals, BrdU incorporation in the last week of the 3-week study was reported to be at a higher baseline level in PPARα-null mice than wild-type mice (~2.5-fold). For wild-type mice the authors reported a statistically significant increase at 500 and 1,000 mg/kg TCE at levels of ~1 and ~4.5% hepatocytes incorporating the label after 5 days of BrdU incorporation. Whether this measure of DNA synthesis is representative of cellular proliferation or of polyploidization was not examined by the authors. Even at 1,000 mg/kg TCE the percent of cells that had incorporated BrdU was less than 5% of hepatocytes in wild-type mice. The magnitude percent liver/body weight ratio change at this exposure level was 4-fold greater than that of hepatocytes undergoing DNA synthesis (16% increase in percent liver/body weight ratio vs. 4% increase in DNA synthesis). The ~1% of hepatocytes undergoing DNA synthesis at the 500 mg/kg TCE level, reported to be statistically significant by the authors, was not correlated with a concurrent increase in percent liver/body weight ratio. Thus, TCE-induced changes in liver weight were not correlated with increases in DNA synthesis in wild-type mice after 3 weeks of TCE exposure. For PPARα-null mice, there was a ~3-fold of control value for the percent of hepatocytes undergoing DNA synthesis at the 1,000 mg/kg TCE exposure level. The higher baseline level in the null mouse, large variability in response at this exposure level, and low power of this experimental design limited the ability to detect statistical significance of this effect although the level was greater than that reported for the 500 mg/kg TCE exposure in wild-type mice that was statistically significant. Thus, TCE appeared to induce an increase in DNA synthesis in PPARα-null mice, albeit at a lower level than wild-type mice. However, the ~2% increase in percent of hepatocytes undergoing DNA synthesis during the 3rd week of a 3-week exposure to 1,000 mg/kg TCE in PPARα-null mice was insufficient to account for the ~10% observed increase in liver weight. For wild-type and PPARα-null mice, the magnitude of TCE-induced increases in liver weight were 4−5-fold higher than that of increases in DNA-synthesis under this paradigm and in both types of mice, a relatively small portion of hepatocytes were undergoing DNA synthesis during the last week of a 3-week exposure duration. Whether the increases in liver weight could have resulted from and early burst of DNA synthesis as well as whether the DNA synthesis results reported here represents either proliferation or polyploidization, cannot be determined from this experiment. Because of the differences in exposure protocol (i.e., use of 3 daily doses in methylcellulose rather than one dose in corn oil) the time course of the transient increase in DNA synthesis reported cannot be assumed to be the same for this experiment and others.

Not only were PPARα-null mice different than wild-type mice in terms of background levels of liver weights, and hepatic steatosis, but this study reported that background levels of PCO activity to be highly variable and in some instances different between wild-type and null mice. There was reported to be ~6-fold PCO activity in PPARα-null control mice in comparison to wild-type control mice in the 1-week DCA/TCA experiment (~0.15 vs. 0.85 units of activity/g protein). However, in the same figure a second set of data are reported for control mice for comparison to WY-14,643 treatment in which PCO activity was slightly decreased in PPARα-null control mice versus wild-type controls (~0.40 vs. 0.65 units of activity/g protein). In the experimental design description of the paper, WY-14,643 treatment and a separate control were not described as part of the 1-week DCA/TCA experiment. For the only experiment in which PCO activity was compared between wild-type and PPARα-null mice exposed to TCE (i.e., 3-day exposure study), there was a reported increased over the control value of ~2.5-fold that was reported to be statistically significant at 1,500 mg/kg TCE (1.5 vs. 0.60 units of activity/g protein). For control mice in the 3-day TCE experiment, there was an increase in this activity in PPARα-null mice in comparison to wild-type mice (~0.60 vs. 0.35 units of activity/g protein). While not statistically significant, there appeared to be a slight increase in PCO activity after 1,500 mg/kg TCE exposure for 3 days in PPARα-null mice of ~30%. However, as noted above the background levels of this enzyme activity varied widely between the experiments with not only values for control animals varying as much as 6-fold (i.e., for PPARα-null mice) but also for WY-14,643 administration. There was a 6.6-fold difference in PCO results for WY-14,643 in PPARα-null mice at the same concentration of WY-14,643 in the 3-day and 1-week experiment, and a 1.44-fold difference in results in wild-type mice in these two data sets.

E.2.1.14. *Ramdhan et al., 2008*

Ramdhan et al. (2008) examined the role of CYP2E1 in TCE-induced hepatotoxicity, using CYP2E1 +/+ (wild-type) and CYP2E1 -/- (null) Sv/129 male mice (6/group) which were exposed for 7 days to 0, 1,000, or 2,000-ppm TCE by inhalation for 8 hours/day (Ramdhan et al., 2008). The exposure concentrations are noted by the authors to be much higher than occupational exposures and to have increased liver toxicity after 8 hours of exposure as measured by plasma AST levels. To put this exposure concentration into perspective, the Kjellstrand et al. (1983a, b) inhalation studies for 30 days showed that these levels were well above the 150-ppm exposure levels in male mice that induced systemic toxicity. Nunes also reported hepatic necrosis up to 4% in rats at 2,000 ppm for just 8 hours not 7 days. AST and ALT were measured at sacrifice. Histological changes were scored using a qualitative scale of 0 = no necrosis, 1 = minimal as defined as only occasional necrotic cells in any lobule, 2 = mild as defined as less than one-third of the lobule structure affected, 3 = moderate as defined as between one-third and two-thirds of the lobule structure affected and 4 = severe defined as greater than two-thirds of the lobule structure affected. Real-time polymerase chain reaction (PCR) was reported for mRNA encoding a number of receptors and proteins. Total RNA and Western Blot analysis was obtained from whole-liver homogenates. The changes in mRNA expression were reported as means for 6 mice per group after normalization to a level of β-actin mRNA expression and were shown relative to the control level in the CYP2E1 wild-type mice.

The deletion of the CYP2E1 gene in the null mouse had profound effects on liver weight. The body were was significantly increased in control CYP2E1 -/- mice in comparison to wild-type controls (24.48 ± 1.44 g for null mice vs. 23.66 ± 2.44 g, m ± SD). This represents a 3.5% increase over wild-type mice. However, the liver weight was reported in the CYP2E1 -/- mice to be 1.32-fold of that of CYP2E1 +/+ mice (1.45 ± 0.10 g vs. 1.10 ± 0.14 g). The percent liver/body weight ratio was 5.47 versus 4.63% or 1.18-fold of wild-type control for the null mice. The authors report that 1,000-ppm and 2,000-ppm TCE treatment did induce a statistically significant change body weight for null or wild-type mice. However, there was an increase in body weight in the wild-type mice (i.e., 23.66 ± 2.44, 24.52 ± 1.17, and 24.99 ± 1.78 for control, 1,000 ppm, and 2,000-ppm groups, respectively) and an increase in the variability in response in the null mice (i.e., 24.48 ± 1.44, 24.55 ± 2.26, and 24.99 ± 4.05, for control, 1,000 ppm, and 2,000 ppm exposure groups, respectively). The percent liver/body weight was 5.47% ± 0.23%, 5.51% ± 0.27%, and 5.58% ± 0.70% for control, 1,000 ppm and 2,000 ppm the CYP2E1 -/- mice, respectively. The percent liver/body weight was 4.63% ± 0.13%, 6.62% ± 0.40%, and 7.24% ± 0.84% for control, 1,000 ppm, and 2,000 ppm wild-type mice, respectively. Therefore, while there appeared to be little difference in the TCE and control exposures for percent liver/body weights in the CYP2E1 -/- mice (2%) there was a 1.56-fold of control level after 2,000 ppm in the wild-type mice after 7 days of inhalation exposure.

The authors reported that "in general, the urinary TCE level in CYP2E1 -/- mice was less than half that in CYP2E1 +/+ mice: urinary TCA levels in the former were about one-fourth those in the latter." Of note is the large variability in urinary TCE detected in the 2,000-ppm TCE exposed wild-type mice, especially after Day 4, and that in general the amount of TCE in the urine appeared to be greatest after the 1st day of exposure and steadily declined between 1 and 7 days (i.e., ~45% decline at 2,000 ppm and a ~70% decline at 1,000 ppm) in the wild-type mice. The amount of TCE in the urine was proportional to the difference in dose at days 1 and 5 (i.e., a 2-fold difference in dose resulted in a 2-fold difference in TCE detected in the urine). As the detection of TCE in the urine declined with time, the amount of TCA was reported to steadily increase between days 1 and 7 (e.g., from ~3 mg TCA after the 1st day to ~5.5 mg after 7 days after 2,000 ppm exposure in wild-type mice). However, unlike TCE, there was a much smaller differences in response between the two TCE exposure levels (i.e., a 12−44% or 1.12- to 1.44-fold difference in TCA levels in the urine at days 1−7 for exposure concentrations that differ by a factor of 2). This could be indicative of saturation in metabolism and TCA clearance into urine at these high concentrations levels. The authors note that their results suggest that the metabolism of TCE in both null and wild-type mice may have reached saturation at 1,000 ppm TCE.

For ALT and AST activities in CYP2E1 -/- or CYP2E1 +/+ mice, both liver enzymes were significantly elevated only at the 2,000 ppm level in CYP2E1 +/+ mice. Although the increases in excreted TCA in the urine differed by only ~33% between the 1,000 and 2,000 ppm levels, liver enzyme levels in plasma differed by a much greater extent after 7 days exposure between the 1,000 and 2,000-ppm groups of CYP2E1 +/+ mice (i.e., 1.26- and 1.83-fold of control [ALT] and 1.40- and 2.20-fold of control [AST] for 1,000 ppm and 2,000 ppm TCE exposure levels, respectively). The authors reported a correlation between plasma ALT and both TCE ($r = 0.7331$) and TCA ($r = 0.8169$) levels but do not report details of what data were included in the correlation (i.e., were data from CYP2E1 +/+ mice combined with those of the CYP2E1 -/- mice and were control values included with treated values?).

The authors show photomicrograph of a section of liver from control CYP2E1 +/+ and CYP2E1 -/- mice and describe the histological structure of the liver to appear normal. This raises the question as to the cause of the hepatomegaly for the CYP2E1 mice in which the liver weight was increased by a third. The qualitative scoring for each of the 6 animals per group showed that none of the CYP2E1 -/- control or treated mice showed evidence of necrosis. For the CYP2E1 +/+ mice there was no necrosis reported in the control mice and in 3/6 mice treated with 1,000 ppm TCE. Of the 3 mice that were reported to have necrosis, the score was reported as 1−2 for 2 mice and 1 for the third. It is not clear what a score of 1−2 represented given the criteria for each score given by the authors, which defined a score of 1 as minimal and one of 2 as mild. For the 2,000 ppm TCE-exposed mice, all mice were reported to have at least minimal necrosis (i.e., 4 mice were reported to have scores of 1−2, one mouse a score of 3 and one mouse a score of 1). What is clear from the histopathology data are that there appeared to be great heterogeneity of response between the 6 animals in each TCE-exposure group in CYP2E1 +/+ mice and that there was a greater necrotic response in the 2,000-ppm-exposed mice than the 1,000 ppm mice. These results are consistent with the liver enzyme data but not consistent with the small difference between the 1,000 ppm and 2,000 ppm exposure groups for TCA content in urine and by analogy, metabolism of TCE to TCA. A strength of this study is that it reports the histological data for each animal so that the heterogeneity of liver response can be observed (e.g., the extent of liver necrosis was reported to range from only occasional necrotic cells in any lobule to between one-third and two-thirds of the lobular structure affected after 2,000 ppm TCE exposure for 7 days). Immunohistochemical analysis was reported to show that CYP2E1 was expressed mainly around the centrilobular area in CYP2E1 +/+ mice where necrotic changes were observed after TCE treatment.

Given the large variability in response within the liver after TCE exposure in CYP2E1 mice, phenotypic anchoring becomes especially important for the interpretation of mRNA expression studies (see Sections E.1.1 and E.3.1.2 for macroarray transcript profiling limitations and the need for phenotypic anchoring). However, the data for mRNA expression of PPARα, peroxisomal bifunctional protein (hydratase+3-hydroxyacyl-CoA dehydrogenase),very long chain acyl-CoA dehydrogenase (VLCAD), CYP4A10, NFκB (p65, P50, P52), and IκBα was reported at the means ± SD for 6 mice per group and represented total liver homogenates. A strength of the study was that they did not pool their RNA and can show means and standard deviations between treatment groups. The low numbers of animals tested however, limits the ability to detect statistically significance of the response. By reporting the means, differences in the responses within dose groups was limited and reflected differential response and involvement for different portions of the liver lobule and for the responses of the heterogeneous group of liver cells populating the liver. The authors reported that they normalized values to the level of β-actin mRNA in same preparation with a value of 1 assigned as the mean from each control group. The values for mRNA and protein expression reported in the figures appeared to have all been normalized to the control values for the CYP2E1 -/- mice. Although all of the CYP2E1 -/- control values were reported as a value of 1, the control values for the CYP2E1+/+ mice differed with the greatest difference being presented for the CYP4A10-mRNA (i.e., the control level of CYP4A10 mRNA was ~3-fold higher in the CYP2E1+/+ mice than the CYP2E1 -/- mice). Further characterization of the CYP2E1 mouse model was not provided by the authors.

The mean expression of PPARα mRNA was reported slightly reduced after TCE treatment in CYP2E1 -/- mice (i.e., 0.72- and 0.78-fold of control after 1,000 and 2,000 ppm TCE exposure, respectively). The CYP2E1 -/- mice had a higher baseline of PPARα mRNA expression than the CYP2E1+/+ mice (i.e., the control level of the CYP2E1 -/- mice was 1.5-fold of the CYP2E1+/+ mice). After TCE exposure, the CYP2E1 +/+ had a similar increase in PPARα mRNA (~2.3-fold) at both 1,000 ppm and 2,000 ppm TCE. Thus, without the presence of CYP2E1 there did not appear to be increased PPARα mRNA expression. For PPARα protein expression, there was a similar pattern with ~1.6-fold of control levels of protein in the CYP2E1 -/- mice after both 1,000 ppm and 2,000 ppm TCE exposures. In the CYP2E1 +/+ mice the control level of PPARα protein was reported to be ~1.5-fold of the CYP2E1 -/- control level. Thus, while the mRNA expression was less, the protein level was greater. After TCE treatment, there was a 2.9-fold of control level of protein at 1,000 ppm TCE and a 3.1-fold of control level of protein at 2,000 ppm. Thus, the magnitude of mRNA increase was similar to that of protein expression for PPARα in CYP2E1 +/+ mice. The magnitude of both was 3-fold or less over control after TCE exposure. This pattern was similar to that of TCA concentration formed in the liver where there was very little difference between the 1,000 and 2,000 ppm exposure groups in CYP2E1 +/+ mice. However, this pattern was not consistent with the liver enzyme and histopathology of the liver that showed a much greater response after 2,000-ppm exposure than 1,000-ppm TCE. In addition, where the mean enzyme markers of liver injury and individual animals displayed marked heterogeneity in response to TCE exposure, there was a much smaller degree of variability in the mean mRNA expression and protein levels of PPARα.

For peroxisomal bifunctional protein there was a greater increase after 1,000 ppm TCE-treated exposure than after 2,000 ppm TCE-treatment for both the CYP2E1 -/- and CYP2E1 +/+ mice (i.e., there was a 2:1 ratio of mRNA expression in the 1,000- vs. 2,000-ppm-exposed groups). The CYP2E1 +/+ mice had a much greater response than the CYP2E1 -/- mice (i.e., the CYP2E1 -/- mice had a 2-fold of control and the CYP2E1 +/+ mice had a 7.8-fold of control level after 1,000 ppm TCE treatment). For peroxisomal bifunctional protein expression, the magnitude of protein induction after TCE exposure was much greater than the magnitude of increase in mRNA expression. In the CYP2E1 -/- mice 1,000 ppm TCE exposure resulted in a 6.9-fold of control level of protein while the 2,000 ppm TCE group had a 2.3-fold level. CYP2E1 +/+ mice had a ~50% higher control level than CYP2E1 mice and after TCE exposure the level of peroxisomal bifunctional protein expression was 44-fold of control at 1,000 ppm TCE and 40-fold of control at 2,000 ppm. Thus, CYP2E1 -/- mice were reported to have less mRNA expression and peroxisomal bifunctional protein formed than CYP2E1 +/+ mice after TCE exposure. However, there appeared to be more mRNA expression after 1,000 ppm than 2,000 ppm TCE in both groups and protein expression in the CYP2E1 -/- mice. After 2,000 ppm TCE, there was similar peroxisomal bifunctional protein expression between the 1,000 ppm and 2,000 ppm TCE treated CYP2E1 +/+ mice. Again this pattern was more similar to that of TCA detection in the urine—not that of liver injury.

For VLCAD the expression of mRNA was similar between control and treated CYP2E1 -/- mice. For CYP2E1 +/+ mice the control level of VLCAD mRNA expression was half that of the CYP2E1 -/- mice. After 1,000 ppm TCE the mRNA level was 3.7-fold of control and after 2,000 ppm TCE the mRNA level was 3.1-fold of control. For VLCAD protein expression was 1.8-fold of control after 1,000 ppm and 1.6-fold of control after 2,000 ppm in CYP2E1 -/- mice. The control level of VLCAD protein in CYP2E1 +/+ mice appeared to be 1.2-fold control CYP2E1 -/- mice. After 1,000-ppm TCE treatment the CYP2E1 -/- mice were reported to have 3.8-fold of control VLCAD protein levels and after 2,000-ppm TCE treatment to have 3.9-fold of control protein levels. Thus, although showing no increase in mRNA there was an increase in VLCAD protein levels that was similar between the two TCE exposure groups in CYP2E1 -/- mice. Both VLCAD mRNA and protein levels were greater in CYP2E1 +/+ mice than CYP2E1 -/- mice after TCE exposure. This was not the case for peroxisomal bifunctional protein. The magnitudes of TCE-induced increases in mRNA and protein increases were similar between the 1,000 and 2,000 ppm TCE exposure concentrations, a pattern more similar to TCA detection in the urine but not that of liver injury.

Finally, for CYP4A10 mRNA expression, there was an increase in expression after TCE treatment of 3-fold for 1,000 ppm and 5-fold after 2,000 ppm in CYP2E1 -/- mice. Thus, although the enzyme assumed to be primarily responsible for TCE metabolism to TCA was missing, there was still a response for the mRNA of this enzyme commonly associated with PPARα activation. Of note is that urinary concentrations of TCA were not zero after TCE exposure in CYP2E1 -/- mice. Both 1,000 and 2,000 ppm TCE exposure resulted in ~0.44 mg TCA after 1 day or about 15−22% of that observed in CYP2E1 +/+ mice. Thus, some metabolism of TCE to TCA is taking place in the null mice, albeit at a reduced rate. For CYP2E1 +/+ mice, 1,000 ppm TCE resulted in an 8.3-fold of control level of CYP4A10 mRNA and 2,000 ppm TCE resulted in a 9.3-fold of control level. The authors did not perform an analysis of CYP4A10 protein. The authors state that "in particular, the mRNA levels of microsomal enzyme CYP4A10 significantly increased in CYP2E1+/+ mice after TCE exposure in a dose-dependent manner." However, the 2-fold difference in TCE exposure concentrations did not result in a similar difference in response as shown above. Both resulted in ~9-fold of control response in CYP2E1 +/+ mice. As with PPARα, peroxisomal bifunctional protein, and VLCAD, the response was more similar to that of TCA detection in the urine and not measured of hepatic toxicity. These data are CYP2E1 metabolism of TCE is important in the manifestation of TCE liver toxicity, however, it also suggests that effects other than TCA concentration and indicators of PPARα are responsible for acute hepatotoxicity resulting from very high concentrations of TCE.

The NFκB family and IκBα were also examined for mRNA and protein expression. These cell signaling molecules are involved in inflammation and carcinogenesis and are discussed in Section E.3.3.3.3 and E.3.4.1.4. Given that presence of hepatocellular necrosis in some of the CYP2E1 +/+ mice to varying degrees, inflammatory cytokines and cell signaling pathways would be expected to be activated. The authors reported that

> overall, TCE exposure did not significantly increase the expression of p65 and p50 mRNAs in either CYP2E1+/+ or CYP2E1 -/- mice… However, p52 mRNA expression significantly increased in the 2,000 ppm group of CYP2E1+/+ mice, and correlation analysis showed that a significant positive relationship existed between the expression of NFκB p52 mRNA and plasma ALT activity.., while no correlation was seen between NFκB p64 or p50 and ALT activity (data not shown).

The authors also note that TCE treatments “did not increase the expression of TNFR1 and TNFR2 mRNA in CYP2E1+/+ and CYP2E1 -/- mice (data not shown).”

A more detailed examination of the data reveals that there was a similar increases in p65, p50, and p52 mRNA expression increases with TCE treatment in CYP2E1 +/+ mice at both TCE exposure levels. However, only p52 levels for the 2,000 ppm-exposed mice were reported to be statistically significant (see comment above about the statistical power of the experimental design and variability between animals). For 1,000 ppm TCE exposure the levels of p65, p50, and p52 mRNA expression were 1.5-, 1.8-, and 2.0-fold of control. For 2,000 ppm TCE the levels of p65, p50, and p52 mRNA expression were 1.8-, 1.8-, and 2.1-fold of control. Thus, there was generally a similar response in all of these indicators of NFκB mRNA expression in CYP2E1 +/+ mice that was mild with little to no difference between the 1,000 ppm and 2,000 ppm TCE exposure levels. For IκBα mRNA expression there was not difference between control and treatment groups for either type of mice. For CYP2E1 -/- mice there appeared to be a ~50% decrease in P52 mRNA expression in mice treated with both exposure concentrations of TCE. The authors plotted the relationship between p52 mRNA and plasma ALT concentration for both CYP2E1 -/- and CYP2E1 +/+ mice together and claimed the correlation coefficient ($r = 0.5075$) was significant. However, of note is that none of the CYP2E1 -/- mice were reported to have either hepatic necrosis or significant increases in ALT detection.

For protein expression, the authors showed results for p50 and p42 proteins. The control CYP2E1 -/- mice appeared to have a slightly lower level of p50 protein expression (~30%) with a much larger increase in p52 protein expression (i.e., 2.1-fold) than CYP2E1 +/+ mice. There appeared to be a 2-fold increase in p50 protein expression after both 1,000-ppm and 2,000 ppm TCE exposures in the CYP2E1 +/+ mice and a similar increase in p52 protein levels (i.e., 1.9- and 2.5-fold of control for 1,000- and 2,000-ppm TCE exposures, respectively). Thus, the magnitude of mRNA and protein levels were similar for p50 and p52 in CYP2E1 +/+ mice and there was no difference between the 1,000- and 2,000-ppm treatments. For the CYP2E1 -/- mice there was a modest increase in p50 protein after TCE exposure (1.1- and 1.3-fold of control for 1,000 and 2,000 ppm respectively) and a slight decrease in p52 protein (0.76- and 0.79-fold of control). There was little evidence that the patterns of either expression or protein production of NFκB family and IκBα corresponded to the markers of hepatic toxicity or that they exhibited a dose-response. The authors note that although he expression of p50 protein increased in CYP2E1 +/+ mice, "the relationship between p50 protein and ALT levels was not significant (data not shown)." For TNFR1 there appeared to be less protein expression in the CYP2E1 +/+ mice than the CYP2E1 -/- mice (i.e., the null mice levels were 1.8-fold of the wild-type mice levels). Treatment with TCE resulted in mild decrease of protein levels in the CYP2E1 -/- mice and a 1.4- and 1.7-fold of control level in the CYP2E1 +/+ mice for 1,000 ppm and 2,000 ppm levels, respectively. For p65, although TCE treatment-related effects were reported, of note the levels of protein were 2.4 higher in the CYP2E1 +/+ mice than the CYP2E1 -/- mice. Thus, protein levels of the NFκB family appeared to have been altered in the knockout mice. Also, as noted in Section E.3.4.1.4, the origin of the NF-κB is crucial as to its effect in the liver and the results of this report are for whole liver homogenates that contain parenchymal as well as nonparenchymal cell and have been drawn from liver that are heterogeneous in the magnitude of hepatic necrosis. The authors suggest that "TCA may act as a defense against hepatotoxicity cause by TCE-delivered reactive metabolite(s) via PPARα in CYP2E1+/+ mice." However, the data from this do not support such an assertion.

E.2.2. Subchronic and Chronic Studies of Trichloroethylene (TCE)

For the purposes of this discussion, studies of duration of 4 weeks or more are considered subchronic. Like those of shorter duration, there is variation in the depth of study of liver changes induced by TCE with many of the longer duration studies focused on the induction of liver cancer. Many subchronic studies were conducted a high doses of TCE that caused toxicity with limited reporting of effects. Similar to acute studies some of the subchronic and chronic studies have detailed examinations of the TCE-induced liver effects while others have reported primarily liver weight changes as a marker of TCE-response. Similar issues also arise with the impact of differences in initial and final body weights between control and treatment groups on the interpretation of liver weight gain as a measure of TCE-response. For many of the subchronic inhalation studies, issues associated with whole body exposures make determination of dose levels difficult. For gavage experiments, death from gavage dosing, especially at higher TCE exposures, is a recurring problem and, unlike inhalation exposures, the effects of vehicle can also be at issue for background liver effects. Concerns regarding effects of oil vehicles, especially corn oil, have been raised with Kim et al. (1990) noting that a large oil bolus will not only produce physiological effects, but alter the absorption, target organ dose, and toxicity of volatile organic compounds (VOCs). Charbonneau et al. (1991) reported that corn oil potentiates liver toxicity from acetone administration that is not related to differences in acetone concentration. Several oral studies in particular document that use of corn oil gavage induces a different pattern of toxicity, especially in male rodents (see Merrick et al., 1989, Section E.2.2.1 below). Several studies listed below report the effects of hepatocellular DNA synthesis and indices of lipid peroxidation (i.e., Channel et al., 1998) are especially subject to background vehicle effects. Rusyn et al. (1999) report that a single dose of dietary corn oil increases hepatocyte DNA synthesis 24 hours after treatment by ~3.5-fold, activation of NF-κB to a similar extent ~2 hours after treatment almost exclusively in Kupffer cells, a ~3−4-fold increase in hepatocytes after 8 hours, and increased in TNFα mRNA between 8 and 24 hours after a single dose in female rats. In regard to studies that have used the i.p. route of administration, as noted by Kawamoto et al. (1988) (see Section E.2.2.10 below), injection of TCE may result in paralytic ileus and peritonitis and that subcutaneous treatment paradigm will result in TCE not immediately being metabolized but retained in the fatty tissue. Wang and Stacey (1990) state that "intraperitoneal injection is not particularly relevant to humans" and that intestinal interactions require consideration in responses such as increase serum bile acid (see Section E.2.3.5 below).

E.2.2.1. *Merrick et al., 1989*

The focus of this study was the examination of potential differences in toxicity or orally gavaged TCE administered in corn oil an aqueous vehicle in B6C3F1 mice. As reported by Melnick et al. (1987) above, corn oil administration appeared to have an effect on peroxisomal enzyme induction. TCE (99.5% purity) was administered in corn oil or an aqueous solution of 20% Emulphor to 14−17 week old mice (n = 12/group) at 0, 600, 1,200 and 2,400 mg/kg/d (males) and 0, 450, 900, and 1,800 mg/kg/d (females) 5 times a week for 4 weeks. The authors state that due to "varying lethality in the study, 10 animals per dose group were randomly selected (where possible) among survivors for histological analysis." Hepatocellular lesions were characterized

> as a collection of approximately 3−5 necrotic hepatocytes surrounded by macrophages and polymorphonuclear cells and histopathological grading was reported as based on the number of necrotic lesions observed in the tissue sections: 0 = normal; 1 = isolated lesions scattered throughout the section; 2 = one to five scattered clusters of necrotic lesions; 3 = more than five scattered clusters of necrotic lesions; and 4 = clusters of necrotic lesions observed throughout the

> entire section." The authors described lipid scoring of each histological section as "0 = no Oil-Red O staining present; 1 = less than 10% staining; 2 = 10-25% staining; 3 = 25-30% staining; and 4 = greater than 50% staining.

The authors reported dose-related increases in lethality in both males and females exposed to TCE in Emulphor with all male animals dying at 2,400 mg/kg/d with 8/12 females dying at 1,800 mg/kg/d. In both males and females, 2/12 animals also died at the next highest dose as well with no unscheduled deaths in control or lowest dose animals. For corn oil gavaged mice, there were 1−2 animals in each TCE treatment groups of male mice that died while there were no unscheduled deaths in female mice. The authors state that lethality occurred within the first week after chemical exposure. The authors present data for final body weight and liver/body weight values for 4 weeks of exposure and list the number of animals per group to be 10−12 for corn oil gavaged animals and the reduced number of animals in the Emulphor gavaged animals reflective of lethality and limiting the usefulness of this measure at the highest doses (i.e., 1,800 mg/kg/d for female mice). In mice treated with TCE in Emulphor gavage, the final body weight of control male animals appeared to be lower than those that were treated with TCE while for female mice the final body weights were similar between treated and control groups. For male mice treated with Emulphor, body weights were 22.8 ± 0.8, 25.3 ± 0.5, and 24.3 ± 0.4 g for control, 600 mg/kg/d, and 1,200 mg/kg/d and for female mice body weights were 20.7 ± 0.4, 21.4 ± 0.3, and 20.5 ± 0.3 g for control, 450 mg/kg/d, and 900 mg/kg/d of TCE.

For percent liver/body weight ratios, male mice were reported to have 5.6% ± 0.2%, 6.6% ± 0.1%, and 7.2% ± 0.2% for control, 600, and 1,200 mg/kg/d and for female mice were 5.1% ± 0.1%, 5.8% ± 0.1%, and 6.5% ± 0.2% for control, 450 mg/kg/d, and 900 mg/kg/d of TCE. These values represent 1.11- and 1.07-fold of control for final body weight in males exposed to 600 and 1,200 mg/kg/d and 1.18- and 1.29-fold of control for percent liver/body weight, respectively. For females, they represent 1.04- and 0.99-fold of control for final body weights in female exposed to 450mg/kg/d and 900 mg/kg/d and 1.14- and 1.27-fold of control for percent liver/body weight, respectively.

In mice treated with corn oil gavage the final body weight of control male mice was similar to the TCE treatment groups and higher than the control value for male mice given Emulphor vehicle (i.e., 22.8 ± 0.8 g for Emulphor control vs. 24.3 ± 0.6 g for corn oil gavage controls or a difference of ~7%). The final body weights of female mice were reported to be similar between the vehicles and TCE treatment groups. The baseline percent liver/body weight was also lower for the corn oil gavage control male mice (i.e., 5.6% for Emulphor vs. 4.7% for corn oil gavage or a difference of ~19% that was statistically significant). Although the final body weights were similar in the female control groups, the percent liver/body weight was greater in the Emulphor vehicle group (5.1% ± 0.1% in Emulphor vehicle group vs. 4.7% ± 0.1% for corn oil gavage or a difference of ~9% that was statistically significant). For male mice treated with corn oil, final body weights were 24.3 ± 0.6, 24.3 ± 0.4, 25.2 ± 0.6, and 25.4 ± 0.5 g for control, 600, 1,200, and 2,400 mg/kg/d, and for female mice body weights were 20.2 ± 0.3, 20.8 ± 0.5, 21.8 ± 0.3 g, and 22.6 ± 0.3 g for control, 450, 900, and 1,800 mg/kg/d of TCE. For percent liver/body weight ratios, male mice were reported to have 4.7% ± 0.1%, 6.4% ± 0.1%, 7.7% ± 0.1%, and 8.5% ± 0.2% for control, 600, 1,200, and 2,400 mg/kg/d and for female mice were 4.7% ± 0.1%, 5.5% ± 0.1%, 6.0% ± 0.2%, and 7.2% ± 0.1% for control, 450, 900, and 1,800 mg/kg/d of TCE. These values represent 1.0-, 1.04-, and 1.04-fold of control for final body weight in males exposed to 600, 1,200, and 2,400 mg/kg/d TCE and 1.36-, 1.64-, and 1.81-fold of control for percent liver/body weight, respectively. For females, they represent 1.03-, 1.08-, and 1.12-fold of control for body weight in female exposed to 450, 900, and 1,800 mg/kg/d and 1.17-, 1.28-, and 1.53-fold of control for percent liver/body weight, respectively.

Because of premature mortality, the difference in TCE treatment between the highest doses that are vehicle-related cannot be determined. The decreased final body weight and increased percent liver/body weight ratios in the Emulphor control animals make comparisons of the exact magnitude of change in these parameters due to TCE exposure difficult to determine as well as differences between the vehicles. The authors did not present data for age-matched controls which did not receive vehicle so that the effects of the vehicles cannot be determined (i.e., which vehicle control values were most similar to untreated controls given that there was a difference between the vehicle controls). A comparison of the percent liver/body weight ratios at comparable doses between the two vehicles shows little difference in TCE-induced liver weight increases in female mice. However, the corn oil vehicle group was reported to have a greater increase in comparison to controls for male mice treated with TCE at the two lower dosage groups. Given that the control values were approximately 19% higher for the Emulphor group, the apparent differences in TCE-dose response may have reflected the differences in the control values rather than TCE exposure. Because controls without vehicle were not examined, it cannot be determined whether the difference in control values was due to vehicle administration or whether a smaller or younger group of animals was studied on one of the control groups. The body weight of the animals was also not reported by the authors at the beginning of the study so that the impact of initial differences between groups versus treatment cannot be accurately determined.

Serum enzyme activities for ALT, AST and LDH (markers of liver toxicity) showed that there was no difference between vehicle groups at comparable TCE exposure levels for male or female mice. Enzyme levels appeared to be elevated in male mice at the higher doses (i.e., 1,200 and 2,400 mg/kg/d for ALT and 2,400 mg/kg/d for AST) with corn oil gavage inducing similar increases in LDH levels at 600, 1,200, and 2,400 mg/kg/d TCE. For ALT and AST there appeared to be a dose-related increase in male mice with the 2,400 mg/kg treatment group having much greater levels than the 1,200 mg/kg group. In Emulphor treatment groups there was a similar increase in ALT levels in males treated with 1,200 mg/kg TCE as with those treated with corn oil and those increases were significantly elevated over control levels. For LDH levels there were similar increase at 1,200 mg/kg TCE for male mice treated using either Emulphor or corn oil. The authors report that visible necrosis was observed in 30−40% of male mice administered TCE in corn oil but not that there did not appear to be a dose-response (i.e., the score for severity of necrosis was reported to be 0, 4, 3, and 4 for corn oil control, 600, 1,200, and 2,400 mg/kg/d treatment groups from 10 male mice in each group). No information in regard to variation between animals was given by the authors. For male mice given Emulphor gavage the extent of necrosis was reported to be 0, 0, and 1 for 0, 600, and 1,200 mg/kg/d TCE exposure, respectively. For female mice, the extent of necrosis was reported to be 0 for all control and TCE treatment groups using either vehicle. Thus, except for LDH levels in male mice exposed to TCE in corn oil there was not a correlation with the extent of necrosis and the increases in ALT and AST enzyme levels. Similarly, there was an increase in ALT levels in male mice treated with 1,200 mg/kg/d exposure to TCE in Emulphor that did not correspond to increased necrosis. For Oil-Red O staining there was a score of 2 in the Emulphor treated control male and female mice while 600 mg/kg/d TCE exposure in Emulphor gavaged male mice and 900 mg/kg/d TCE in corn oil gavaged female mice had a score of 0, along with the corn oil gavage controls in male mice. For female control mice treated with corn oil gavage, the staining was reported to have a score of 3. Thus, there did not appear to be a dose-response in Oil-Red oil staining although the authors claimed there appeared to be a dose-related increase with TCE exposure. The authors described lesions produced by TCE exposure as

> focal and were surrounded by normal parenchymal tissue. Necrotic areas were not localized in any particular regions of the lobule. Lesions consisted of central necrotic cells encompassed by hepatocytes with dark eosinophilic staining cytoplasm, which progressed to normal-appearing cells. Areas of necrosis were accompanied by localized inflammation consisting of macrophages and polymorphonuclear cells.

No specific descriptions of histopathology of mice given Emulphor were provided in terms of effects of the vehicle or TCE treatment. The scores for necrosis was reported to be only a 1 for the 1,200 mg/kg concentration of TCE in male mice gavaged with Emulphor but 3 for male mice given the same concentration of TCE in corn oil. However, enzyme levels of ALT, AST, and LDH were similarly elevated in both treatment groups.

These results do indicate that administration of TCE for 4 weeks via gavage using Emulphor resulted in mortality of all of the male mice and most of the female mice at a dose in corn oil that resulted in few deaths. Not only was there a difference in mortality, but vehicle also affected the extent of necrosis and enzyme release in the liver (i.e., Emulphor vehicle caused mortality as the highest dose of TCE in male and female mice that was not apparent from corn oil gavage, but Emulphor and TCE exposure induced little if any focal necrosis in males at concentrations of TCE in corn oil gavage that caused significant focal necrosis). In regard to liver weight and body weight changes, TCE exposure in both vehicles at nonlethal doses induced increased percent liver/body weight changes male and female mice that increased with TCE exposure level. The difference in baseline control levels between the two vehicle groups (especially in males) make a determination of the quantitative difference vehicle had on liver weight gain problematic although the extent of liver weight increase appeared to be similar between male and female mice given TCE via Emulphor and female mice given TCE via corn oil. In general, enzymatic markers of liver toxicity and results for focal hepatocellular necrosis were not consistent and did not reflect dose-responses in liver weight increases. The extent of necrosis did not correlate with liver weight increases and was not elevated by TCE treatment in female mice treated with TCE in either vehicle, or in male mice treated with Emulphor. There was a reported difference in the extent of necrosis in male mice given TCE via corn oil and female mice given TCE via corn oil but the necrosis did not appear to have a dose-response in male mice. Female mice given corn oil and male and female mice given TCE in Emulphor had no to negligible necrosis although they had increased liver weight from TCE exposure.

E.2.2.2. ***Goel et al., 1992***

The focus of this study was the description of TCE exposure related changes in mice after 28 days of exposure with regard to TCE-induced pathological and liver weight change. Male Swiss mice (20−22 g body weight or 9% difference) were exposed to 0, 500, 1,000 or 2,000 mg/kg/d TCE (BDH analytical grade) by gavage in groundnut oil (n = 6 per group) 5 days a week for 28 days. The ages of the mice were not given by the authors. Livers were examined for “free -SH contents,” total proteins, catalase activity, acid phosphatase activity, and “protein specific for peroxisomal origin of approx, 80 kd.” The authors report no statistically significant change in body weight with TCE treatment but a significant increase in liver weight. Body weight (mean ± SE) was reported to be 32.67 ± 1.54, 31.67 ± 0.61, 33.00 ± 1.48, and 27.80 ± 1.65 g from exposure to oil control, 500, 1,000, and 2,000 mg/kg/d TCE, respectively.

There was a 15% decrease in body weight at the highest exposure concentration of TCE that was not statistically significant, but the low number of animals examined limits the power to detect a significant change. The percent relative liver/body weight was reported to be 5.29% ± 0.48%, 7.00% ± 0.36%, 7.40% ± 0.39%, and 7.30% ± 0.48% from exposure to oil control, 500, 1,000, and 2,000 mg/kg/d TCE, respectively. This represents 1.32-, 1.41-, and 1.38-fold of control in percent liver/body weight for 500, 1,000, and 2,000 mg/kg/d TCE, respectively. The "free –SH content" in μmol −SH/g tissue was reported to be 5.47 ± 0.17, 7.46 ± 0.21, 7.84 ± 0.34, and 7.10 ± 0.34 from exposure to oil control, 500, 1,000, and 2,000 mg/kg/d TCE, respectively. This represents 1.37-, 1.44-, and 1.30-fold of control in –SH/g tissue weight for 500, 1,000, and 2,000 mg/kg/d TCE, respectively. Total protein content in the liver in mg/g tissue was reported to be 170 ± 3, 183 ± 5, 192 ± 7, and 188 ± 3 from exposure to oil control, 500, 1,000, and 2,000 mg/kg/d TCE, respectively. This represents 1.08-, 1.13-, and 1.11-fold of control in total protein content for 500, 1,000, and 2,000 mg/kg/d TCE, respectively. Thus, the increases in liver weight, "free -SH content" and increase protein content were generally parallel and all suggest that liver weight increases had reached a plateau at the 1,000 mg/kg/d exposure concentration perhaps reflecting toxicity at the highest dose as demonstrated by decreased body weight in this study.

The enzyme activities of δ-ALA dehydrogenase ("a key enzyme in heme biosynthesis"), catalase, and acid phosphatase were assayed in liver homogenates. Treatment with TCE decreased δ-ALA dehydrogenase activity to a similar extent at all exposure levels (32−35% reduction). For catalase the activity as units of catalase/mg protein was reported to be 25.01 ± 1.81, 32.46 ± 2.59, 41.11 ± 5.37, and 33.96 ± 3.00 from exposure to oil control, 500, 1,000, and 2,000 mg/kg/d TCE, respectively. This represents 1.30-, 1.64-, and 1.36-fold in catalase activity for 500, 1,000, and 2,000 mg/kg/d TCE, respectively. The increasing variability in response with TCE exposure concentration is readily apparent from these data as is the decrease at the highest dose, perhaps reflective of toxicity. For acid phosphatase activity in the liver there was a slight increase (5−11%) with TCE exposure that did not appear to be dose-related.

The authors report that histologically, "the liver exhibits swelling, vacuolization, widespread degeneration/necrosis of hepatocytes as well as marked proliferation of endothelial cells of hepatic sinusoids at 1000 and 2000 mg/kg TCE doses." Only one figure is given at the light microscopic level in which it is impossible to distinguish endothelial cells from Kupffer cells and no quantitative measures or proliferation were examined or reported to support the conclusion that endothelial cells are proliferating in response to TCE treatment. Similarly, no quantitation regarding the extent or location of hepatocellular necrosis is given. The presence or absence of inflammatory cells is not noted by the authors as well. In terms of white blood cell count, the authors note that it is slightly increased at 500 mg/kg/d but decreased at 1,000 and 2,000 mg/kg/d TCE, perhaps indicating macrophage recruitment from blood to liver and kidney, which was also noted to have pathology at these concentrations of TCE.

E.2.2.3. *Kjellstrand et al., 1981*

This study was conducted in mice, rats, and gerbils and focused on the effects of 150-ppm TCE exposure via inhalation on body and organ weight. No other endpoints other than organ weights were examined in this study and the design of the study is such that quantitative determinations of the magnitude of TCE response are very limited. NMRI mice (weighing ~30 g with age not given), S-D rats (weighing ~200 g with age not given, and Mongolian gerbils (weighing ~60 g with age not given) were exposed to 150-ppm TCE continuously. Mice were exposed for 2, 5, 9, 16, and 30 days with the number of exposed animals and controls in the 2, 5, 9, and 16 days groups being 10. For 30-day treatments there were two groups of mice containing 20 mice per group and one group containing 12 mice per group. In addition there was a group of mice (n = 15) exposed to TCE for 30 days and then examined 5 days after cessation of exposure and another group (n = 20) exposed to TCE for 30 days and then examined 30 days after cessation of exposure. For rats there were three groups exposed to TCE for 30 days, which contained 24, 12, and 10 animals per group. For gerbils there were three groups exposed to TCE for 30 days, which contained 24, 8, and 8 animals per group. The groups were reported to consist of equal numbers of males and female but for the mice exposed to TCE for 30 days and then examined 5 days later, the number was 10 males and 5 females. Body weights were reported to be recorded before and after the exposure period. However, the authors state "for technical reasons the animals within a group were not individually identified, i.e., we did not know which initial weight in the group corresponded to which final one." They authors state that this design presented problems in assessing the precision of the estimate. They go on to state that rats and gerbils were partially identifiable as the animals were housed 3 to a cage and cage averages could be estimated. Not only were mice in one group housed together but

> even worse: at the start of the experiment, the mice in M2 [group exposed for 2 days] and M9 [group exposed for 9 days] were housed together, and similarly M5 [group exposed for 5 days] and M16 [group exposed for 16 days]. Thus, we had, e.g., 10 initial weights for exposed female mice in M2 and M9 where we could not identify those 5 that were M2 weights. Owing to this bad design (forced upon us by the lack of exposure units), we could not study weight gains for mice and so we had to make do with an analysis of final weights.

The problems with the design of this study are obvious from the description given by the authors themselves. The authors state that they assumed that the larger the animal the larger the weight of its organs so that all organ weights were converted into relative weights as percentage of body weight. The fallacy of this assumption is obvious, especially if there was toxicity that decreased body weight and body fat but at the same time caused increased liver weight as has been observed in many studies at higher doses of TCE. In fact, Kjellstrand et al. (1983b) report that a 150-ppm TCE exposure for 30 days does significantly decreases body weight while elevating liver weight in a group of 10 male NMRI mice. Thus, the body weight estimates from this study are inappropriate for comparison to those in studies where body weights were actually measured. The liver/body weight ratios that would be derived from such estimates of body weights would be meaningless. The group averages for body weight reported for female mice at the beginning of the 30-day exposure varied significantly and ranged from 23.2 to 30.2 g (~24%). For males, the group averages ranged from 27.3 to 31.4 g (~14%). For male mice there was no weight estimate for the animals that were exposed for 30 days and then examined 30 days after cessation of exposure.

The authors only report relative organ weight at the end of the experiment rather than the liver weights for individual animals. Thus, these values represent extrapolations based on to what body weight may have been. For mice that were exposed to TCE for 30 days and the examined after 30 days of exposure, male mice were reported to have "relative organ weight" for liver of 4.70% ± 0.10% versus 4.27% ± 0.13% for controls. However, there were no initial body weights reported for these male mice and the body weights are extrapolated values. Female mice exposed for 30 days and then examined 30 days after cessation of exposure were reported to have "relative organ weights" for liver of 4.42% ± 0.11% versus 3.62% ± 0.09%. The group average of initial body weights for this group was reported by the authors. Although the initial body weight for female control mice as a group average was reported to be similar between the female group exposed to 30 days of TCE and sacrificed 30 days later and those exposed for 30 days and sacrificed 5 days later (30.0 g vs. 30.8 g), the liver/body weight ratio varied significantly in these controls (4.25 ± 0.19 vs. 3.62 ± 0.09) as did the number of animals studied (5 female mice in the animals sacrificed after 5 days exposure versus 10 female mice in the group sacrificed after 30 days exposure). In addition, although there were differences between the 3 groups of mice exposed to TCE for 30 days and then sacrificed immediately, the authors present the data for extrapolated liver/body weight as pooled results between the 3 groups. In comparison to control values, the authors report 1.14-, 1.35-, 1.58-, 1.47-, and 1.75-fold of control for percent liver/body weight using body weight extrapolated values in male mice at 2, 5, 9, 16, and 30 days of TCE exposure, respectively. For females, they report 1.27-, 1.28-, 1.49-, 1.41-, and 1.74-fold of control at 2, 5, 9, 16, and 30 days of TCE, respectively. Although the authors combine female and male relative increases in liver weight in a figure, assign error bars around these data point, and attempt to draw assign a time-response curve to it, it is clear from these data, especially for female mice, do not display time-dependent increase in liver/body weight from 5 to 16 days of exposure and that a comparison of results between 5 animals and 26 is very limited in interpretation. Of note is the wide variation in the control values for relative liver/body weight. For male mice there did not seem to be a consistent pattern with increasing duration of the experiment with values at 4.61, 5.15, 5.05, 4.93, and 4.04% for 2, 5, 9, 16, and 30-day exposure groups. This represented a difference of ~27%. For female mice, the relative liver/body weight was 4.14, 4.58, 4.61, 4.70, and 3.99% for 2, 5, 9, 16, and 30 day exposure groups. Thus, it appears that the average relative liver/body weight percent was higher in the 5, 9, and 16 day treatment group for both genders than that to the 30 day group and was consistent between these days. There is no apparent reason for there to be such large difference between 16 day and 30-day treatment groups due to increasing age of the animals. Of note is that for the control groups pared with animals treated for 30 days and then examined 30 days later, the male mice had increases in relative liver/body weight (4.27 vs. 4.04%) but that the females had a decrease (3.62 vs. 3.99%). Such variation between controls does not appear to be age and size related but to variations in measure or extrapolations, which can affect comparisons between treated and untreated groups and add more uncertainty to the estimates.

The number of mice in the groups exposed to 2 though 16 days were only 5 animals for each gender in each group while the number of animals reported in the 30-day exposure group numbered 26 for each gender.

For animals exposed to 30 days and then examined after 5 or 30 days, male mice were reported to have percent liver/body weight 1.26- and 1.10-fold of control after 5 and 30 days cessation of exposure while female mice were reported to have values of 1.14- and 1.22-fold of control after 5 and 30 days cessation of exposure, respectively. Again, the male mice exposed for 30 days and then examined after 30 days of cessation of exposure did not have reported initial body weights giving this value a great deal of uncertainty. Thus, while liver weights appeared to increase during 30 days of exposure to TCE and decreased after cessation of exposure in both genders of mice, the magnitudes of the increases and decrease cannot be determined from this experimental design. Of note is that liver weights appeared to still be elevated after 30 days of cessation exposure.

In regard to initial weights, the authors report that the initial weight of the rats were different in the 3 experiments they conducted with them and state that “in those 2 where differences were found in females, their initial weights were about 200 g and 220 g, respectively, while the corresponding weights were only about 160 g in that experiment where no differences were found." The differences in initial body weight of the rat groups were significant. In females group averages were 198, 158, and 224 g, for groups 1, 2, and 3, respectively, and for males group averages were 222, 166, and 248 g for groups 1,2, and 3 respectively. This represents as much as a 50% difference in initial body weights between these TCE treatment groups. Control values varied as well with group averages for controls ranging from167 g for group 2 to 246 g for group 3 at the start of exposure. For female rats control groups ranged from 158 to 219 g at the start of the experiment. The number of animals in each group varied greatly as well making quantitative comparison even more difficult with the numbers varying between 5 and 12 for each gender in rats exposed for 30 days to TCE. The authors pooled the results for these very disparate groups of rats in their reporting of relative organ weights. They reported 1.26- and 1.21-fold of control in male and female rat percent relative liver/body weight after 30 days of TCE exposure. However, as stated above, these estimates are limited in their ability to provide a quantitative estimate of liver weight increase due to TCE.

There were evidently differences between the groups of gerbils in response to TCE with one group reported to have larger weight gain than control and the other 2 groups reported to not show a difference by the authors. Of the 3 groups of gerbils, group 1 contained 12 animals per gender but groups 2 and 3 only 4 animals per gender. As with the rat experiments, the initial average weights for the groups varied significantly (30% in females and males). The authors pooled the results for these very disparate groups of gerbils in their reporting of relative organ weights as well. They reported a nearly identical increase in relative liver/body weight increase for gerbil (1.22-fold of control value in males and 1.25-fold in females) as for the rat after 30 days of TCE exposure. However, similar caveats should be applied in the confidence in this experimental design to determine the magnitudes of response to TCE exposure.

E.2.2.4. *Woolhiser et al., 2006*

An unpublished report by Woolhiser et al. (2006) was received by the U.S. EPA to fill the "priority data needed" for the immunotoxicity of TCE as identified by the Agency for Toxic Substances and Disease Registry and designed to satisfy U.S. EPA OPPTS 870.7800 Immunotoxicity Test Guidelines. The study was conducted on behalf of the Halogenated Solvents Industry Alliance and has been submitted to the U.S. EPA but not published. Although conducted as an immunotoxicity study, it does contain information regarding liver weight increases in female Sprague Dawley (S-D) female rats exposed to 0, 100, 300, and 1,000 ppm TCE for 6 hours/day, 5 days/week for 4 weeks. The rats were 7 weeks of age at the start of the study. The report gives data for body weight and food weight for 16 animals per exposure group and the mean body weights ranged between 181.8 to 185.5 g on the first day of the experiment. Animals were weight pre-exposure, twice during the first week, and then "at least weekly throughout the study." All rats were immunized with a single intravenous injection of sheep red blood cells via the tail vein at Day 25. Liver weights were taken and samples of liver retained "should histopathological examination have been deemed necessary." But, histopathological analysis was not conducted on the liver.

The effect on body weight gain by TCE inhalation exposure was shown by 5 days and continued for 10 days of exposure in the 300-ppm and 1,000-ppm-exposed groups. By Day 28, the mean body weight for the control group was reported to be 245.7 g but 234.4 g, 232.4 g, and 232.4 g for the 100-ppm, 300-ppm, and 1,000-ppm exposure groups, respectively. Food consumption was reported to be decreased in the day1−5 measurement period for the 300-and 1,000-ppm exposure groups and in the 5−10 day measurement period for the 100-ppm group. Although body weight and food consumption data are available for 16 animals per exposure group, for organ and organ/body weight summary data, the report gives information for only 8 rats per group. The report gives individual animal data in its appendix so that the data for the 8 animals in each group examined for organ weight changes could be examined separately. The final body weights were reported to be 217.2, 212.4, 203.9, and 206.9 g for the control, 100-, 300-, and 1,000-ppm exposure groups containing only 8 animals. For the 8-animal exposure groups, the mean initial body weights were 186.6, 183.7, 181.6, and 181.9 g for the control, 100-, 300-, and 1,000-ppm exposure groups. Thus, there was a difference from the initial and final body weight values given for the groups containing 16 rats and those containing 8 rats. The ranges of initial body weights for the eight animals were 169.8−204.3, 162.0−191.2, 169.0−201.5, and 168.2−193.7 g for the control, 100-, 300 -, and 1,000-ppm groups. Thus, the control group began with a larger mean value and large range of values (20% difference between highest and lowest weight rat) than the other groups.

In terms of the percent liver/body weight ratios, an increase due to TCE exposure is reported in female rats, although body weights were larger in the control group and the two higher exposure groups did not gain body weight to the same extent as controls. The mean percent liver/body weight ratios were 3.23, 3.39, 3.44, and 3.65%, respectively for the control, 100-ppm, 300-ppm, and 1,000-ppm exposure groups. This represented 1.05-, 1.07-, and 1.13-fold of control percent liver/body weight changes in the 100-, 300-, and 1,000-ppm exposure groups. However, the small number of animals and the variation in initial animal weight limit the ability of this study to determine statistically significant increases and the authors report that only the 1,000-ppm group had statistically significant increased liver weight increases.

E.2.2.5. *Kjellstrand et al., 1983a*

This study examined seven strains of mice (wild, C57BL, DBA, B6CBA, A/sn, NZB, and NMRI) after continuous inhalation exposure to 150-ppm TCE for 30 days. "Wild" mice were reported to be composed of "three different strains: 1. Hairless (HR) from the original strain, 2. Swiss (outbred), and 3. Furtype Black Pelage (of unknown strain)." The authors do not state the age of the animals prior to TCE exposure but state that weight-matched controls were exposed to air only chambers. The authors state that "the exposure methods" have been described earlier (Kjellstrand et al., 1980) but only reference Kjellstrand et al. (1981). In both of this and the 1981 study, animals were continuously exposed with only a few hours of cessation of exposure noted a week for change of food and bedding. Under this paradigm, there is the possibility of additional oral exposure to TCE due to grooming and consumption of TCE on food in the chamber. The study was reported to be composed of two independent experiments with the exception of strain NMRI which had been studied in Kjellstrand et al. (1981, 1983b). The number of animals examined in this study ranged from 3−6 in each treatment group. The authors reported "significant difference between the animals intended for TCE exposure and the matched controls intended for air-exposure were seen in four cases (Table 1.)," and stated that the grouping effects developed during the 7-day adaptation period. Premature mortality was attributed to an accident for one TCE-exposed DBA male and fighting to the deaths of two TCE-exposed NZB females and one B6CBA male in each air exposed chamber. Given the small number of animals examined in this study in each group, such losses significantly decrease the power of the study to detect TCE-induced changes. The range of initial body weights between the groups of male mice for all strains was between 18 g (as mean value for the A/sn strain) and 32 g (as mean value for the B6CBA strain) or ~44%. For females, the range of initial body weights between groups for all strains was 15 g (as mean value for the A/sn strain) and 24 g (as mean value for the DBA strain) or ~38%.

Rather than reporting percent liver/body weight ratios or an extrapolated value, as was done in Kjellstrand et al. (1981), this study only reported actual liver weights for treated and exposed groups at the end of 30 days of exposure. The authors report final body weight changes in comparison to matched control groups at the end of the exposure periods but not the changes in body weight for individual animals. They report the results from statistical analyses of the difference in values between TCE and air-exposed groups. A statistically significant decrease in body weight was reported between TCE exposed and control mice in experiment 1 of the C57BL male mice (~20% reduction in body weight due to TCE exposure). This group also had a slight but statistically significant difference in body weight at the beginning of exposure with the control group having a ~5% difference in starting weight. There was also a statistically significant decrease in body weight of 20% reported after TCE exposure in one group of male B6CBA mice that did not have a difference in body weight at the beginning of the experiment between treatment and control groups. One group of female and both groups of male A/sn mice had statistically significant decreases in body weight after TCE exposure (10% for the females, and 22 and 26% decreases in the two male groups) in comparison to untreated mice of the same strain. The magnitude of body weight decrease in this strain after TCE treatment also reflects differences in initial body weight as there were also differences in initial body weight between the two groups of both treated and untreated A/sn males that were statistically significant, 17 and 10% respectively. One group of male NZB mice had a significant increase in body weight after TCE exposure of 14% compared to untreated animals. A female group from the same strain treated with TCE was reported to have a nonsignificant but 7% increase in final body weight in comparison to its untreated group. The one group of male NMRI mice ($n = 10$) in this study was reported to have a statistically significant 12% decrease in body weight compared to controls.

For the groups of animals with reported TCE exposure-related changes in final body weight compared to untreated animals, such body weight changes may also have affected the liver weights changes reported. The authors do not explicitly state that they did not record liver and body weights specifically for each animal, and thus, would be unable to determine liver/body weight ratios for each, however, they do state that he animals were housed 4−6 in each cage and placed in exposure chambers together. The authors only present data for body and liver weights as the means for a cage group in the reporting of their results. While this approach lends more certainty in their measurements than the approach taken by Kjellstrand et al. (1981) as described above, the relative liver/body weights cannot be determined for individual animals. It appears that the authors have tried to carefully match the body weights of the control and exposed mice at the beginning of the experiment to minimize the effects of initial body weight differences and distinguish the effects of treatment on body weight and liver weight. However, there is no ability to determine liver/body weight ratios and adjust for difference in initial body weight from changes due to TCE exposure. For the groups in which there was no change in body weight after TCE treatment and in which there was no difference in initial body weight between controls and TCE-exposed groups, the reporting of liver weight changes due to TCE exposure is a clearer reflection of TCE-induced effects and the magnitude of such effects. Nevertheless the small number of animals examined in each group is still a limitation on the ability to determine the magnitude of such responses and there statistical significance.

In wild-type mice there were no reported significant differences in the initial and final body weight of male or female mice before or after 30 days of TCE exposure. For these groups there was 1.76- and 1.80-fold of control values for liver weight in groups 1 and 2 for female mice, and for males 1.84- and 1.62-fold of control values for groups 1 and 2, respectively. For DBA mice there were no reported significant differences in the initial and final body weight of male or female mice before or after 30 days of TCE exposure. For DBA mice there was 1.87- and 1.88-fold of control for liver weight in groups 1 and 2 for female mice, and for males 1.45- and 2.00-fold of control for groups 1 and 2, respectively. These groups represent the most accurate data for TCE-induced changes in liver weight not affected by initial differences in body weight or systemic effects of TCE, which resulted in decreased body weight gain. These results suggest that there is more variability in TCE-induced liver weight gain between groups of male than female mice.

The C57BL, B6CBA, NZB, and NMRI groups all had at least one group of male mice with changes in body weight due to TCE exposure. The A/sn group not only had both male groups with decreased body weight after TCE exposure (along with differences between exposed and control groups at the initiation of exposure) but also a decrease in body weight in one of the female groups. Thus, the results for TCE-induced liver weight change in these male groups also reflect changes in body weight. These results suggest a strain-related increased sensitivity to TCE toxicity as reflected by decreased body weight. For C57BL mice, there was 1.65- and 1.60-fold of control for liver weight after TCE exposure was reported in groups 1 and 2 for female mice, and for males 1.28-fold (the group with decreased body weight) and 1.82-fold of control values for groups 1 and 2, respectively. For B6CBA mice there was 1.70- and 1.69-fold of controls values for liver weight after TCE exposure in groups 1 and 2 for female mice, and for males 1.21-fold (the group with decreased body weight) and 1.47-fold of control values reported for groups 1 and 2, respectively. For the NZB mice there was 2.09-fold (n = 3) and 2.08-fold of control values for liver weight after TCE exposure in groups 1 and 2 for female mice and for males 2.34- and 3.57-fold (the group with increased body weight) of control values reported for groups 1 and 2, respectively. For the NMRI mice, whose results were reported for one group with 10 mice, there was 1.66-fold of control value for liver weight after TCE exposure for female mice and for males 1.68-fold of control value reported (a group with decreased body weight). Finally, for the A/sn strain that had decreased body weight in all groups but one after TCE exposure and significantly smaller body weights in the control groups before TCE exposure in both male groups, the results still show TCE-related liver weight increases. For the As/n mice there was 1.56- and 1.72-fold (a group with decreased body weight) of control value for liver weight in groups 1 and 2 for female mice and for males 1.62-fold (a group with decreased body weight) and 1.58-fold (a group with decreased body weight) of control values reported for groups 1 and 2, respectively.

The consistency between groups of female mice of the same strain for TCE-induced liver weight gain, regardless of strain examined, is striking. The largest difference within female strain groups occurred in the only strain in which there was a decrease in TCE-induced body weight. For males, even in strains that did not show TCE-related changes in body weight, there was greater variation between groups than in females. For strains in which one group had TCE-related changes in body weight and another did not, the group with the body weight decrease always had a lower liver weight as well. Groups that had increased body weight after TCE exposure also had an increased liver weight in comparison to the groups without a body weight change. These results demonstrate the importance of carefully matching control animals to treated animals and the importance of the effect of systemic toxicity, as measured by body weight decreases, on the determination of the magnitude of liver weight gain induced by TCE exposure. These results also show the increased variation in TCE-induced liver weight gain between groups of male mice and an increase incidence of body weight changes due to TCE exposure in comparison to females, regardless of strain.

In terms of strain sensitivity, it is important not only to take into account differing effects on body weight changes due to TCE exposure but also to compare animals of the same age or beginning weight as these parameters may also affect liver weight gain or toxicity induced by TCE exposure. The authors do not state the age of the animals at the beginning of exposure and report, as stated above, a range of initial body weights between the groups as much as 44% for males and 38% for females. These differences can be due to strain and age. The differences in final body weight between the groups of controls, when all animals would have been 30 days older and more mature, was still as much as 48% for males and 44% for females. The data for female mice, in which body weight was decreased by TCE exposure only in on group in one strain, suggest that the magnitude of TCE-induced liver weight increase was correlated with body weight of the animals at the beginning of the experiment. For the C57BL and As/n strains, female mice starting weights were averaged 17.5 and 15.5 g, respectively, while the average liver weights were 1.63- and 1.64-fold of control after TCE exposure, respectively. For the B6CBA, wild-type, DBA, and NZB female groups the starting body weights averaged 22.5, 21.0, 23.0, and 21.0 g, respectively, while the average liver weight increases were 1.70-, 1.78-, 1.88-, and 2.09-fold of control after TCE exposure. Thus, groups of female mice with higher body weights, regardless of strain, generally had higher increases in TCE-induced liver weight increases. The NMRI group of female mice, did not follow this general pattern and had the highest initial body weight for the single group of 10 mice reported (i.e., 27 g) associated with a 1.66-fold of control value for liver weight. It is probable that the data for these mice had been collected from another study. In fact, the starting weights reported for these groups of 10 mice are identical to the starting weights reported for 26 mice examined in Kjellstrand et al. (1981). However, while this study reports a 1.66-fold of control value for liver weight after 30 days of TCE exposure, the extrapolated percent liver/body weight given in the 1981 study for 30 days of TCE exposure was 1.74-fold of control in female NMRI mice. In the Kjellstrand et al. (1983b) study, discussed below, 10 female mice were reported to have a 1.66-fold of control value for liver weight after 30 days exposure to 150-ppm TCE with an initial starting weight of 26.7 g. Thus, these data appear to be from that study. Thus, differences in study design, variation between experiments, and strain differences may account for the differences results reported in Kjellstrand et al. (1983a) for NMRI mice and the other strains in regard to the relationship to initial body weight and TCE response of liver weight gain.

These data suggest that initial body weight is a factor in the magnitude of TCE-induced liver weight induction rather than just strain. For male mice, there appeared to be a difference between strains in TCE-induced body weight reduction, which in turn affects liver weight. The DBA and wild-type mice appeared to be the most resistant to this effect (with no groups affected), while the C57BL, B6CBA, and NZB strains appearing to have at least one group affected, and the A/sn strain having both groups of males affected. Only one group of NMRI mice were reported in this study and that group had TCE-induced decreases in body weight. As stated above there appeared to be much greater differences between groups of males within the same strain in regard to liver weight increases than for females and that the increases appeared to be affected by concurrent body weight changes. In general the strains and groups within strain, that had TCE-induced body weight decreases, had the smallest increases in liver weight, while those with no TCE-induced changes in body weight in comparison to untreated animals (i.e., wild-type and DBA) or had an actual increase in body weight (one group of NZB mice) had the greatest TCE-induced increase in liver weight. Therefore, only examining liver weight in males rather than percent liver/body weight ratios would not be an accurate predictor of strain sensitivity at this dose due to differences in initial body weight and TCE-induced body weight changes.

E.2.2.6. *Kjellstrand et al., 1983b*

This study was conducted in male and female NMRI mice with a similar design as Kjellstrand et al. (1983a). The ages of the mice were not given by the authors. Animals were housed 10 animals per cage and exposed from 30 to 120 days at concentrations ranging from 37 to 3,600 ppm TCE. TCE was stabilized with 0.01% thymol and 0.03% diisopropylene. Animals were exposed continuously with exposure chambers being opened twice a week for change of bedding food and water resulting in a drop in TCE concentration of ~1 hour. A group of mice was exposed intermittently with TCE at night for 16 hours. This paradigm results not only in inhalation exposure but, also, oral exposure from TCE adsorption to food and grooming behavior. The authors state that "the different methodological aspects linked to statistical treatment of body and organ weights have been discussed earlier (Kjellstrand et al., 1981). The same air-exposed control was used in three cases." The design of the experiment, in terms of measurement of individual organ and body weights and the inability to assign a percent liver/body weight for each animal, and limitations are similar to that of Kjellstrand et al. (1983b). The exposure design was for groups of male and female mice to be exposed to 37-, 75-, 150-, and 300-ppm TCE continuously for 30 days (*n* = 10 per gender and group except for the 37 ppm exposure groups) and then for liver weight and body weight to be determined. Additional groups of animals were exposed for 150 ppm continuously for 120 days (*n* = 10). Intermittent exposure of 4 hours/day for 7 days a week were conducted for 120 days at 900 ppm and examined immediately or 30 days after cessation of exposure (*n* = 10). Intermittent exposures of 16 hours/day at 255-ppm group (*n* = 10), 8 hours/day at 450 ppm, 4 hours/day at 900 ppm, 2 hours/day at 1,800 ppm, and 1 hour/day at 3,600 ppm 7 days/week for 30 days were also conducted (*n* = 10 per group).

As in Kjellstrand et al. (1983a), body weights for individual animals were not recorded in a way that the initial and final body weights could be compared. The approach taken by the authors was to match the control group at the initiation of exposure and compare control and treated average values. At the beginning of the experiment only one group began the experiment with a statistically significant change in body weight between treated and control animals (female mice exposed 16 hours a day for 30 days). In regard to final body weight, which would indicate systemic TCE toxicity, 5 groups had significantly decreased body weight (i.e., males exposed to 150 ppm continuously for 30 or 120 days, males and females exposed continuously to 300 ppm for 30 days) and 2 groups significantly increased body weight (i.e., males exposed to 1,800 ppm for 2 hours/day and 3,600 ppm for 1 hour/day for 30 days) after TCE exposure. Thus, the accuracy of determining the effect of TCE on liver weight changes, reported by the authors in this study for groups in which body weight were also affected by TCE exposure, would be affected by similar issues as for data presented by Kjellstand et al. (1983a). In addition, comparison in results between the 37-ppm exposure groups and those of the other groups would be affected by difference in number of animals examined (10 vs. 20). As with Kjellstrand et al (1983a), the ages of the animals in this study are not given by the author. Difference in initial body weight (which can be affected by age and strain) reported by Kjellstrand et al. (1983a) appeared to be correlated with the degree of TCE-induced change in liver weight. Although each exposed group was matched to a control group with a similar average weight, the average initial body weights in this study varied between groups (i.e., as much as 14% in female control, 16% in TCE-exposed female mice, 12% in male control, and 16% in male exposed mice).

For female mice exposed from 37 ppm to 300 ppm TCE continuously for 30 days, only the 300 pm group experienced a 16% decrease in body weight between control and exposed animals. Thus, liver weight increased reported by this study after TCE exposure were not affected by changes in body weight for exposures below 300 ppm in female mice. Initial body weights in the TCE-exposed female mice were similar in each of these groups (i.e., range of 29.2−31.6 g, or 8%), with the exception of the females exposed to 150 ppm TCE for 30 days (i.e., initial body weight of 27.3 g), reducing the effects of differences in initial body weight on TCE-induced liver weight induction. Exposure to TCE continuously for 30 days resulted in a dose-dependent change in liver weight in female mice with 1.06-, 1.27-, 1.66-, and 2.14-fold of control values reported for liver weight at 37 ppm, 75 ppm, 150 ppm, and 300 ppm TCE, respectively. In females, the increase at 300 ppm was accompanied by statistically significant decreased body weight in the TCE exposed groups compared to control (~16%). Thus, the response in liver weight gain at that exposure is in the presence of toxicity. However, the TCE-induced increases in liver weight consistently increased with dose of TCE in a linear fashion.

For male mice exposed to 37 to 300 ppm TCE continuously for 30 days, both the 150- and 300-ppm-exposed groups experienced a 10 and 18% decrease in body weight after TCE exposure, respectively. The 37- and 75-ppm groups did not have decreased body weight due to TCE exposure but varied by 12% in initial body weight. Thus, there are more factors affecting reported liver weight increases from TCE exposure in the male than female mice, most importantly toxicity. Exposure to TCE continuously for 30 days resulted in liver weights of 1.15-, 1.50-, 1.69-, and 1.90-fold of control for 37, 75, 150, and 300 ppm, respectively. The flattening of the dose-response curve for liver weight in the male mice is consistent with the effects of toxicity at the two highest doses, and thus, the magnitude of response at these doses should be viewed with caution. Consistent with Kjellstrand et al. (1983a) results, male mice in this study appeared to have a higher incidence of TCE-induced body weight changes than female mice.

The effects of extended exposure, lower durations of exposure but at higher concentrations, and of cessation of exposure were examined for 150 ppm and higher doses of TCE. Mice exposed to TCE at 150 ppm continuously for 120 days were reported to have increased liver weight (i.e., 1.57-fold of control for females and 1.49-fold of control for males), but in the case of male mice, also to have a significant decrease in body weight of 17% in comparison to control groups. Increasing the exposure concentration to 900-ppm TCE and reducing exposure time to 4 hours/day for 120 days also resulted in increased liver weight (i.e., 1.35-fold of control for females and 1.49-fold of controls for males) but with a significant decrease in body weight in females of 7% in comparison to control groups. For mice that were exposed to 150-ppm TCE for 30 days and then examined 120 days after the cessation of exposure, liver weights were 1.09-fold of control for female mice and the same as controls for male mice. With the exception of 1,800 ppm and 3,600 ppm TCE groups exposed at 2 and 1 hour, respectively, exposure from 225 ppm, 450 and 900 ppm at 16, 8, and 4 hours, respectively for 30 days did not result in decreased body weight in males or female mice. These exposures did result in increased liver weights in relation to control groups and for female mice the magnitude of increase was similar (i.e., 1.50-, 1.54-, and 1.51-fold of control for liver weight after exposure to 225-ppm TCE 16 hours/day, 450-ppm TCE 8 hours/day, and 900-ppm TCE 4 hours/day, respectively). For these groups, initial body weights varied by 13% in females and 14% in males. Thus, under circumstances without body weight changes due to TCE toxicity, liver weight appeared to have a consistent relationship with the product of duration and concentration of exposure in female mice. For male mice, the increases in TCE-induced liver weight were more variable (i.e., 1.94-, 1.74-, and 1.61-fold of control for liver weight after exposure to 225-ppm TCE 16 hours/day, 450-ppm TCE 8 hours/day, and 900-ppm TCE 4 hours/day, respectively) with the product of exposure duration and concentration did not result in a consistent response in males (e.g., a lower dose for a longer duration of exposure resulted in a greater response than a larger dose at a shorter duration of exposure).

Kjellstrand et al. (1983b) reported light microscopic findings from this study and report that

> after 150 ppm exposure for 30 days, the normal trabecular arrangement of the liver cells remained. However, the liver cells were generally larger and often displayed a fine vacuolization of the cytoplasm. The nucleoli varied slightly to moderately in size and shape and had a finer, granular chromatin with a varying basophilic staining intensity. The Kupffer cells of the sinusoid were increased in cellular and nuclear size. The intralobular connective tissue was infiltrated by inflammatory cells. There was not sign of bile stasis. Exposure to TCE in higher or lower concentrations during the 30 days produced a similar morphologic picture. After intermittent exposure for 30 days to a time weighted average concentration of 150 ppm or continuous exposure for 120 days, the trabecular cellular arrangement was less well preserved. The cells had increased in size and the variations in size and shape of the cells were much greater. The nuclei also displayed a greater variation in basophilic staining intensity, and often had one or two enlarged nucleoli. Mitosis was also more frequent in the groups exposed for longer intervals. The vacuolization of the cytoplasm was also much more pronounced. Inflammatory cell infiltration in the interlobular connective tissue was more prominent. After exposure to 150 ppm for 30 days, followed by 120

days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes.

Although not reporting comparisons between changes in male and female mice in the results section of the paper, the authors state in the discussion section that "However, liver mass increase and the changes in liver cell morphology were similar in TCE-exposed male and female mice."

The authors do not present any quantitative data on the lesions they describe, especially in terms of dose-response. Most of the qualitative description is for the 150-ppm exposure level, in which there are consistent reports of TCE induced body weight decreases in male mice. The authors suggest that lower concentrations of TCE give a similar pathology as those at the 150-ppm level, but do not present data to support that conclusion. Although stating that Kupffer cells were increased in cellular and nuclear size, no differential staining was applied light microscopy sections distinguish Kupffer from endothelial cells lining the hepatic sinusoid in this study. Without differential staining such a determination is difficult at the light microscopic level. Indeed, Goel et al. (1992) describe proliferation of sinusoidal endothelial cells after 1,000 mg/kg/d and 2,000 mg/kg/d TCE exposure for 28 days in male Swiss mice. However, the described inflammatory cell infiltrates in the Kjellstrand et al. (1983b) study are consistent with invasion of macrophages and well as polymorphonuclear cells into the liver, which could activate resident Kupffer cells. Although not specifically describing the changes as consistent with increased polyploidization of hepatocytes, the changes in cell size and especially the continued change in cell size and nuclear staining characteristics after 120 days of cessation of exposure are consistent with changes in polyploidization induced by TCE. Of note is that in the histological description provided by the authors, although vacuolization is reported and consistent with hepatotoxicity or lipid accumulation, which is lost during routine histological slide preparation, there is no mention of focal necrosis or apoptosis resulting from these exposures to TCE.

E.2.2.7. *Buben and O'Flaherty, 1985*

This study was conducted with older mice than those generally used in chronic exposure assays (Male Swiss-Cox outbred mice between 3 and 5 months of age) with a weight range reported between 34 to 45 g. The mice were administered distilled TCE in corn oil by gavage 5 times a week for 6 weeks at exposure concentrations of either 0, 100, 200, 400, 800, 1,600, 2,400, or 3,200 mg TCE/kg/day. While 12−15 mice were used in most exposure groups, the 100- and 3,200-mg/kg groups contained 4−6 mice and the two control groups consisted of 24 and 26 mice. Liver toxicity was determined by "liver weight increases, decreases in liver glucose-6-phosphate (G6P) activity, increases in liver triglycerides, and increases in serum glutamate-pyruvate transaminase (SGPT) activity." Livers were perfused with cold saline prior to testing for weight and enzyme activity and hepatic DNA was measured.

The authors reported the mice to tolerate the 6-week exposed with TCE with few deaths occurring except at the highest dose and that such deaths were related to central nervous system depression. Mice in all dose groups were reported to continue to gain weight throughout the 6-week dosing period. However, TCE exposure caused "dose-related increases in liver weight to body weight ratio and since body weight of mice were generally unaffected by treatment, the increases represent true liver weight increases." Exposure concentrations, as low as 100 mg/kg/d, were reported to be "sufficient to cause statistically significant increase in the liver weight/body weight ratio," and the increases in liver size to be "attributable to hypertrophy of the liver cells, as revealed by histological examination and by a decrease in the DNA concentration in the livers." Mice in the highest dose group were reported to display liver weight/body weight ratios that were about ~75% greater than those of controls and even at the lowest dose there was a statistically significant increase (i.e., control liver/body weight percent was reported to be 5.22% ± 0.09% vs. 5.85% ± 0.20% in 100 mg/kg/d exposed mice). The percent liver/body ratios were 5.22% ± 0.09%, 5.84% ± 0.20%, 5.99% ± 0.13%, 6.51% ± 0.12%, 7.12% ± 0.12%, 8.51% ± 0.20%, 8.82% ± 0.15%, and 9.12% ± 0.15% for control (n = 24), 100 (n = 5), 200 (n = 12), 400 (n = 12), 800 (n = 12), 1,600 (n = 12), 2,400 (n = 12), and 3,200 (n = 4) mg/kg/d TCE. This represents 1.12-, 1.15-, 1.25-, 1.36-, 1.63-, 1.69-, and 1.75-fold of control for these doses. All dose groups of TCE induced a statistically significant increase in liver/body weight ratios. For the 200 through 1,600 mg/kg exposure levels, the magnitudes of the increases in TCE exposure concentrations were similar to the magnitudes of TCE-induced increases in percent liver/body weight ratios (i.e., a ~2-fold increase in TCE dose resulted in ~1.7-fold increase change in percent liver/body weight).

TCE exposure was reported to induce a dose-related trend towards increased triglycerides (i.e., control values of 3.08 ± 0.29 vs. 6.89 ± 1.40 at 2,400 mg/kg TCE) with variation of response increased with TCE exposure. For liver triglycerides the reported values in mg/g liver were 3.08 ± 0.29 (n = 24), 3.12 ± 0.49 (n = 5), 4.41 ± 0.76 (n = 12), 4.53 ± 1.05 (n = 12), 5.76 ± 0.85 (n = 12), 5.82 ± 0.93 (n = 12), 6.89 ± 1.40 (n = 12), and 7.02 ± 0.69 (n = 4) for control, 100, 200, 400, 800, 1,600, 2,400, and 3,200 mg/kg/d dose groups, respectively.

For G6P the values in μg phosphate/mg protein/20 minutes were 125.5 ± 3.2 (n = 12), 117.8 ± 6.0 (n = 5), 116.4 ± 2.8 (n = 9), 117.3 ± 4.6 (n = 9), 111.7 ± 3.3 (n = 9), 89.9 ± 1.7 (n = 9), 83.8 ± 2.1 (n = 8), and 83.0 ± 7.0 (n = 3) for the same dose groups. Only the 2,400 mg/kg/d dosing group was reported to be statistically significantly increased for triglycerides after TCE exposure although there appeared to be a dose-response. For decreases in G6P the 800 mg/kg/d and above doses were statistically significant. The numbers of animals varied between groups in this study but in particular only a subset of the animals were tested for G6P with the authors providing no rationale for the selection of animals for this assay. The differences in the number of animals per group and small number of animals per group affected the ability to determine a statistically significant change in these parameters but the changes in liver weights were robust enough and variation small enough between groups that all TCE-induced changes were described as statistically significant. The livers of TCE treated mice, although enlarged, were reported to appear normal. A dose-related decrease in glucose-6-phophatase activity was reported with similar small decreases (~10%) observed in the TCE exposed groups that did not reach statistical significance until the dose reached 800 mg/kg TCE exposure. SGPT activity was not observed to be increased in TCE-treated mice except at the two highest doses and even at the 2,400 mg/kg dose half of the mice had normal values. The large variability in SGPT activity was indicative of heterogeneity of this response between mice at the higher exposure levels for this indicator of liver toxicity. However, the results of this study also demonstrate that hepatomegaly was a robust response that was observed at the lowest dose tested, was dose-related, and was not accompanied by toxicity.

Liver histopathology and DNA content were determined only in control, 400, and 1,600 mg/kg TCE exposure groups. DNA content was reported to be significantly decreased from 2.83 ± 0.17 mg/g liver in controls to 2.57 ± 0.14 in 400 mg/kg TCE treated group, and to 2.15 ± 0.08 mg/kg liver in the 1,600 mg/kg exposed group. This result was consistent with a decreased number of nuclei per gram of liver and hepatocellular hypertrophy. Liver degeneration was reported as swollen hepatocytes and to be common with treatment. "Cells had indistinct borders; their cytoplasm was clumped and a vesicular pattern was apparent. The swelling was not simply due to edema, as wet weight/dry weight ratios did not increase." Karyorhexis (the disintegration of the nucleus) was reported to be present in nearly all specimens and suggestive of impending cell death. A qualitative scale of negative, 1, 2, 3, or 4 was given by the authors to rate their findings without further definition or criterion given for the ratings. "No Karyorhexis, necrosis, or polyploidy was reported in controls, but a score of 1 for Karyorhexis was given for 400 mg/kg TCE and 2 for 1600 mg/kg TCE." Central lobular necrosis reported to be present only at the 1,600 mg/kg TCE exposure level and as a score of 1. "Polyploidy was also characteristic in the central lobular region" with a score of 1 for both 400 and 1,600 mg/kg TCE. The authors reported that "hepatic cells had two or more nuclei or had enlarged nuclei containing increased amounts of chromatin, suggesting that a regenerative process was ongoing" and that there were no fine lipid droplets in TCE exposed animals. The finding of “no polyploidy” in control mouse liver is unexpected given that binucleate and polyploid hepatocytes are a common finding in the mature mouse liver. It is possible that the authors were referring to unusually high instances of “polyploidy” in comparison to what would be expected for the mature mouse. The score given by the authors for polyploidy did not indicate a difference between the two TCE exposure treatments and that it was of the lowest level of severity or occurrence. No score was given for centrolobular hypertrophy although the DNA content and liver weight changes suggested a dose response. The “Karyorhexis” described in this study could have been a sign of cell death associated with increased liver cell number or dying of maturing hepatocytes associated with the increased ploidy, and suggests that TCE treatment was inducing polyploidization. Consistent with enzyme analyses, centrilobular necrosis was only seen at the highest dose and with the lowest qualitative score, indicating that even at the highest dose there was little toxicity.

Thus, the results of this study of TCE exposure for 6 weeks, is consistent with acute studies and show that the region of the liver affected by TCE is the centralobular region, that hepatocellular hypertrophy is observed in that region, and that increased liver weight is induced at the lowest exposure level tested and much lower than those inducing overt toxicity. These authors suggest polyploidization is occurring as a result of TCE exposure although a quantitative dose response cannot be determined from these data.

E.2.2.8. *Channel et al., 1998*

This study was performed in male hybrid B6C3F1/CrlBR mice (13 weeks-old, 25−30 grams) and focused on indicators of oxidative stress. TCE was administered by oral gavage 5 days a week in corn oil for up to 55 days for some groups. Although the study design indicated that water controls, corn oil controls, and exposure levels of 400, 800, and 1,200 mg/kg day TCE in corn oil, results were not presented for water controls for some parameters measured. Initial body weights and those recorded during the course of the study were not reported for individual treatment groups. Liver samples were collected on study days 2, 3, 6, 10, 14, 21, 28, 35, 42, 49, and 56. Histopathology was studied from a single section taken from the median lobe. Thiorbarbiturate acid-reactive substances (TBARS) were determined from whole liver homogenates. Nuclei were isolated from whole liver homogenates and DNA assayed for 8-hydroxy-2’ deoxyguanosine (8-OHdG). There was no indication that parenchymal cell and nonparenchymal cells were distinguished in the assay. Free radical electron paramagnetic resonance (EPR) for total radicals was analyzed in whole liver homogenates. For peroxisome detection and analysis, livers from 3 mice from the 1,200 mg/kg TCE and control (oil and water) groups were analyzed via electron microscopy. Only centrilobular regions, the area stated by the authors to be the primary site of peroxisome proliferation, were examined. For each animal, 7 micrographs of randomly chosen hepatocytes immediately adjacent to the central vein were examined with peroxisomal area to cytoplasmic area, the number of peroxisomes per unit area of cytoplasm, and average peroxisomal size quantified. Proliferation cell nuclear antigen (PCNA), described as a marker of cell cycle except G0, was examined in histological sections for a minimum of 18 fields per liver section. The authors did not indicate what areas of the liver lobule were examined for PCNA. Apoptosis was detected on liver sections using a apoptosis kit using a single liver section from the median lobe and based on the number of positively labeled cells per 10 mm^2 in combination with the morphological criteria for apoptosis of Columbano et al. (1985). However, the authors did not indicate what areas of the liver lobule were specifically examined.

The authors reported that body weight gain was not adversely affected by TCE dosing of the time course of the study but did not show the data. No gross lesions were reported to be observed in any group. For TBARS no water control data was reported by the authors. Data were presented for 6 animals per group for the corn oil control group and the 1,200 mg/kg group (error bars representing the SE). No data were presented without corn oil so that the effects of corn oil on the first day of the study (Day 2 of dosing) could not be determined. After 2 and 3 days of dosing the corn oil and 1,200-mg/kg TCE groups appeared to have similar levels of TBAR detected in whole liver as nmol TBARS/mg protein. However, by Day 6 the corn oil treated control had a decrease in TBAR that continued until Day 15 where the level was ~50% of that reported on Days 2 and 3. The variation between animals as measured by SE was reported to be large on Day 10. By Day 20 there was a slight increase in variation that declined by Day 35 and stayed the same through Day 55. For the TCE exposed group the TBARs remained relatively consistent and began to decline by about Day 20 to a level that similar to the corn oil declines by Day 35. Therefore, corn oil alone had a significant effect on TBAR detection inducing a decline by 6 days of administration that persisted thought 55 days. TCE administration at the 1,200 mg/kg dose in corn oil appeared to have a delayed decline in TBARS. The authors interpreted this pattern to show that lipid peroxidation was elevated in the 1,200 mg/kg TCE group at Day 6 over corn oil. However, corn oil alone induced a decrease in TBARs. At no time was TBARS in TCE treatment groups reported to be greater than the initial levels at days 2 and 3, a time in which TCE and corn oil treatment groups had similar levels. Rather than inducing increasing TBARS over the time course of the study TCE, at the 1,200 mg/kg dose, appeared to delay the corn oil induced suppression of TBARs detection. Because the authors did not present data for aqueous control animals, the time course of TBARS detection in the absence of corn oil, cannot be established.

For the 800 and 400 mg/kg TCE data the authors presented a figure, without standard error information, for up to 35 days that shows little difference between 400 mg/kg TCE treatment and corn oil suppression of TBAR induction. There was little difference between the patterns of TBAR detection for 800 and 400 mg/kg TCE, indicating that both delayed TBAR suppression by corn oil to a similar extent and did not induce greater TBAR than corn oil alone.

For 8-OHdG levels, the authors report that elevations were modest with the greatest increase noted in the 1,200 mg/kg day TCE treatment group of 196% of oil controls on Day 56. Levels fluctuated throughout the study with most of the time points that were elevated showing 129% of control for the 1,200 mg/kg/d group. Statistically significant elevations were noted on days 2, 10, 28, 49 and 56 with depression on Day 3. On all other days (i.e., Days 6, 14, 21, 35, and 42) the 8-OHdG values were similar to those of corn oil controls. No statistically significant effects were reported to be observed at lower doses. The figure presented by the authors shows the percent of controls by TCE treatment at 1,200 mg/kg/d but not the control values themselves. The pattern by corn oil is not shown and neither is the standard error of the data. As a percent of control values the variations were very large for many of the data points and largest for the data given at Day 55 in which the authors report the largest difference between control and TCE treatment. There was no apparent pattern of elevation in 8-OHdG when the data were presented in this manner. Because the data for the corn oil control was not given, as well as no data given for aqueous controls, the effects of corn oil alone cannot be discerned.

Given that for TBARS corn oil had a significant effect and showed a pattern of decline after 6 days, with TCE showing a delayed decline, it is especially important to discern the effects of corn oil and to see the pattern of the data. At time points when TBARS levels were reported to be the same between corn oil and TCE (Days 42, 49 and 56) the pattern of 8-OHdG was quite different with a lower level at Day 42 a slightly increased level at Day 49 and the highest difference reported at Day 56 between corn oil control and TCE treated animals. The authors report that the pattern of "lipid peroxidation" to be similar between the 1,200 and 800 mg/kg doses of TCE but for there to be no significant difference between 800 mg/kg TCE and corn oil controls. Thus, the pattern of TBARS as a measure of lipid peroxidation and 8-OHdG level in nuclear DNA did not match.

In regard to total free radical levels as measured by EPR, results were reported for the 1,200 mg/kg TCE as a signal that was subtracted from control values with the authors stating that only this dose level induced an elevation significantly different from controls. Again, aqueous control values were not presented to discern the effects of corn oil or the pattern that may have arisen with time of corn oil administration. The pattern of total free radical level appeared to differ from that of lipid peroxidation and for that of 8-OHdG DNA levels with no changes at days 2, 3, a peak level at Day 6, a rapid drop at Day 10, mild elevation at Day 20, and a significant decrease at Day 49. The percentage differences between control and treated values reported at Day 6 and 20 by the authors was not proportional to the fold-difference in signal indicating that there was not a consistent level for control values over the time course of the experiment. While differences in lipid peroxidation detection between 1,200 mg/kg TCE and corn oil control were greatest at Day 14, total free radicals showed their biggest change between corn oil controls and TCE exposure on Day 6, time points in which 8-OHdG levels were similar between TCE treatment and corn oil controls. Again, there was no reported difference between corn oil control and the 800 mg/kg TCE exposed group in total free radical formation but for lipid peroxidation the 800 mg/kg TCE exposed group had a similar pattern as that of 1,200 mg/kg TCE.

Only the 1,200 mg/kg group was evaluated for peroxisomal proliferation at days 6, 10, and 14. Thus, correlations with peroxisome proliferation and other parameters in the report at differing times and TCE exposure concentrations could not be made. The authors report that there was a treatment and time effect for percent peroxisomal area, a "treatment only" effect for number of peroxisome and no effect for peroxisomal size. They also report that hepatocytes examined from corn oil control rats were no different that those from water control rats for all peroxisomal parameter, thus, discounting a vehicle effect. However, there was an effect on peroxisomal size between corn oil control and water with corn oil decreasing the peroxisomal size in comparison to water on all days tested. The highest TCE-induced percent peroxisomal area and number occurred on Day 10 of the 3 time points measured for this dose and the fold increase was ~4.5- and ~3.1-fold increase, respectively. The day-10 peak in peroxisomal area and number does not correlate with the reported pattern of free radical or 8-OHdG generation.

For cell proliferation and apoptosis, data were given for days 2, 6, 10, 14, and 21 in a figure. PCNA cells, a measure of cells that have undergone DNA synthesis, was elevated only on Day 10 and only in the 1,200 mg/kg/d TCE exposed group with a mean of ~60 positive nuclei per 1,000 nuclei for 6 mice (~6%). Given that there was little difference in PCNA positive cells at the other TCE doses or time points studied, the small number of affected cells in the liver could not account for the increase in liver size reported in other experimental paradigms at these doses. The PCNA positive cells as well as "mitotic figures" were reported to be present in centrilobular, midzonal, and periportal regions with no observed predilection for a particular lobular distribution. No data were shown regarding any quantitative estimates of mitotic figures and whether they correlated with PCNA results. Thus, whether the DNA synthesis phases of the cell cycle indicated by PCNA staining were indentifying polyploidization or increased cell number cannot be determined. The authors reported that there was no cytotoxicity manifested as hepatocellular necrosis in any dose group and that there was no significant difference in apoptosis between treatment and control groups with data not shown. The extent of apoptosis in any of the treatment groups, or which groups and timepoints were studied for this effect cannot be determined. No liver weight or body weight data were provided in this study.

These results confirm that as a vehicle corn oil is not neutral in its affects in the liver. The TBARS results indicate a reduction in detection of TBARS in the liver with increasing time of exposure to corn oil alone. Although control animals "treated with water" gavage were studied, only the results for peroxisome proliferation were presented by the study so that the effects of corn oil gavage were not easy to discern. In addition, the data were presented in such a way for 8-OHdG and total free radical changes that the pattern of corn oil administration was obscured. It is not apparent from this study that TCE exposure induces oxidative damage.

E.2.2.9. *Dorfmueller et al., 1979*

The focus of this study was the evaluation of "teratogenicity and behavioral toxicity with inhalation exposure of maternal rats" to TCE. Female Long-Evans hooded rats (n = 12) of ~210 g weight were treated with 1,800 ± 200-ppm TCE for 6 hours/day, 5 days/week, for 22 ± 6 days (until pregnancy confirmation) continuing through Day 20 of gestation. Control animals were exposed 22 ± 3 days before pregnancy confirmation. The TCE used in this study contained 0.2% epichlorhydrin. Body weights were monitored as well as maternal liver weight at the end of exposure. Other than organ weight, no other observations regarding the liver were reported in this study. The initial weights of the dams were 212 ± 39 g (mean ± SD) and 204 ± 35 g for treated and control groups, respectively. The final weights were 362 ± 32 g and 337 ± 48 g for treated and control groups, respectively. There was no indication of maternal toxicity by body weight determinations as a result of TCE exposure in this experiment and there was also no significant difference in absolute or relative percent liver/body weight between control and treated female rats in this study.

E.2.2.10. *Kumar et al., 2001*

In this study, adult male Wistar rats (130 ± 10 g body weight) were exposed to 376 ± 1.76 ppm TCE ("AnalaR grade") for 8, 12, and 24 weeks for 4 hours/day 5 days/week. The ages of the rats were not given by the authors. Each group contained 6 rats. The animals were exposed in whole body chambers and thus, additional oral exposure was probable. Along with histopathology of light microscopic sections, enzymatic activities of alkaline phosphatase and acid phosphatase, glutamic oxoacetate transaminase, glutamic pyruvate transaminase, reduced glutathione and "total sulphydryl" were assayed in whole liver homogenates as well as total protein. The authors state that "the size and weight of the liver were significantly increased after 8, 12, and 24 weeks of TCE exposure." However, the authors do not report the final body weight of the rats after treatment nor do they give quantitative data of liver weight changes. In regard to histopathology, the authors state

> After 8 weeks of exposure enlarged hepatocytes, with uniform presence of fat vacuoles were found in all of the hepatocytes affecting the periportal, midzonal, and centrilobular areas, and fat vacuoles pushing the pyknosed nuclei to one side of hepatocytes. Moreover congestion was not significant. After exposure of 12 and 24 weeks, the fatty changes became more progressive with marked necrosis, uniformly distributed in the entire organ.

No other description of pathology was provided in this report. In regard to the description of fatty change, the authors only do conventional H&E staining of sections with no precautions to preserve or stain lipids in their sections. The authors provide a table with histological scoring of simply + or – for minimal, mild or moderate effects and do not define the criteria for that scoring. There is also no quantitative information given as to the extent, nature, or location of hepatocellular necrosis. The authors report "no change was observed in GOT and GPT levels of liver in all the three groups. The GSH level was significantly decreased while TSH level was significantly increased during 8, 12, and 24 weeks of TCE exposure. The acid and alkaline phosphatases were significantly increased during 8, 12, and 24 weeks of TCE exposure." The authors present a series of figures that are poor in quality to demonstrate histopathological TCE-induced changes. No mortality was observed from TCE exposure in any group despite the presence of liver necrosis.

E.2.2.11. *Kawamoto et al., 1988*

The focus of this study was the long-term effects of TCE treatment on induction of metabolic enzymes in male adult Wistar rats. The authors reported that 8 rats weighing 200 g were treated with 2.0 g/kg TCE in olive oil administered subcutaneously twice a week for 15 weeks with 7 rats serving as olive oil controls. In a separate experiment, 5 rats were injected with 1.0 g/kg TCE in olive oil i.p. once a day for 5 continuous days. For comparative purposes groups of 5 rats each were administered 3-methylcholanthrene (20 mg/kg in olive oil i.p.), Phenobarbital (80 mg/kg in saline i.p.) for 4 days as well as ethanol administered in drinking water containing 10% ethanol for 14 days. Microsomes were prepared one week after the last exposure from rats administered TCE for 15 weeks and 24 hours after the last exposure for the other treatments.

Body weights were reported to be slightly less for the TCE treated group than for controls with the initial weights, shown in a figure, to be similar for the first weeks of exposure. At 15 weeks there appeared to be ~7.5% difference in mean body weights between control and TCE treated rats which the authors reported to not be significantly different. Organ weights at the termination of the experiment were reported to only be different for the liver with a 1.21-fold of control value reported as a percentage of body weight with TCE treatment. The authors report their increase in liver weights in male rats from subcutaneous exposure to TCE in olive oil (2.0 g/kg) to be consistent with the range of liver weight gain in rats reported by Kjellstrand et al. (1981) for 150-ppm TCE inhalation exposure (see comments on that study above). The 5-day i.p. treatment with TCE was also reported to only produce increased liver weight but the data were not shown and the magnitude of the percentage increase was not given by the authors. No liver pathology results were studied or reported as well.

Along with an increase in liver weight, 15-week treatment with TCE was reported to cause a significant increase of microsomal protein/g liver of ~20% (10.64 ± 0.88 vs. 12.58 ± 0.71 mg/g liver for olive oil controls and TCE treatment, respectively). Microsomal cytochrome P450 content was reported to show a mild increase that was not statistically significant of 1.08-fold (1.342 ± 0.205 vs. 1.456 ± 0.159 nmol/mg protein for olive oil controls and TCE treatment, respectively) of control. However, cytochrome P450 content showed 1.28-fold of control value (14.28 ± 2.41 vs. 18.34 ± 2.31 nmol/g liver for olive oil controls and TCE treatment, respectively) in terms of g/liver. Chronic treatment of TCE was also reported to cause a significant increase in cytochrome b-5 level (~1.35-fold of control) and NADPH-cytochrome c reductase activity (~1.50-fold of control) in g/liver.

The 5-day TCE treatment via the i.p. route of administration was reported to cause a significant increase in microsomal protein (~20%), induce cytochrome P450 (~50% increase g/liver and 22% increase in microsomal protein), but to also increase cytochrome b-5 and NADPH-cytochrome c reductase activity by 50 and 70% in g/liver, respectively. Although weaker, 5-day i.p. treatment with TCE induced an enzyme pattern more similar to that of Phenobarbital and ethanol rather methylcholanthrene (i.e., increased cytochrome P450 but not microsomal protein and NADPH-cytochrome c reductase). Direct quantitative comparisons of vehicle effects and potential impact on response to TCE treatments for 15 weeks subcutaneous exposure and 5-day i.p. exposure could not be made as baseline levels of all enzyme and protein levels changed as a function of age. Of note is that, in the discussion section of the paper, the authors disclose that injection of TCE 2.0 or 3.0 g/kg i.p. for 5 days resulted in paralytic ileus from TCE exposure as unpublished observations. They note that the rationale for injecting TCE subcutaneously was not only that it did not require an inhalation chamber but also guarded against peritonitis that sometimes occurs following repeated i.p. injection. In terms of comparison with inhalation or oral results, the authors note that the subcutaneous treatment paradigm will result in TCE not immediately being metabolized but retained in the fatty tissue and that after cessation of exposure TCE metabolites continued to be excreted into the urine for more than 2 weeks.

E.2.2.12. *National Toxicology Program (NTP), 1990*

E.2.2.12.1. ***13-week studies.*** The NTP conducted a 13 weeks study of 7 week old F344/N rats (10 rats per group) that received doses of 125 to 2,000 mg/kg (males [0, 125, 250, 500, 1,000, or 2,000 mg/kg]) and 62.5 to 1,000 mg/kg (females [0, 62.5, 125, 250, 500, or 1,000 mg/kg] TCE via corn oil gavage 5 days per week (see Table E-1). For 7-week old B6C3F1mice (n = 10 per group), the dose levels were reported to be 375 to 6,000 mg/kg TCE (0, 375, 750, 1,500, 3,000, or 6,000 mg/kg). Animals were exposed via corn oil gavage to TCE that was epichlorhydrin free. All rats were reported to survive the 13-week study, but males receiving 2,000 mg/kg exhibited a 24% difference in final body weight. However, there was great variation in initial weights between the dose groups with mean initial weights at the beginning of the study reported to 87, 88, 92, 95, 101, and 83 grams for the control, 125, 250, 500, 1,000, and 2,000 mg/kg dose groups in male rats, respectively. This represents a 22% difference between the highest and lowest initial weights between groups. Thus, changes in final body weight after TCE treatment also reflect differences in starting weights between the groups which in the case of the 500, and 1,000 mg/kg groups would results in an lower than expected change in weight due to TCE exposure. For female rats, the mean initial starting weights were reported to be 81, 72, 74, 75, 73, and 76 g, respectively for the control, 62.5, 125, 250, 500, and 1,000 mg/kg dose groups. This represents a ~13% difference between initial weights. In the case of female rats the larger mean initial weight in the control group would tend to exaggerate the effects of TCE exposure on final body weight. The authors did not report the variation in initial or final body weights within the dose groups. At the lowest doses for male and female rats body mean weights were reported to be decreased by 6 and 7% in male and female rats, respectively. Organ weight changes were not reported for rats.

For male mice, mean initial body weights ranged from 19 to 22 g (~16% difference) and for female mice ranged between 18 and 15 g (20% difference), and thus, similar to rats, the final body weights in the groups dose with TCE reflect not only the effects of the compound but also differences in initial weights. For male mice, the mean final body weights were reported to be 3 to 17% less than controls for the 375 to 3,000 mg/kg dose. For female mice the percent difference in final body weight was reported to be the same except for the 6,000 mg/kg dose group but this lack of difference between controls and treated female mice reflected no change in mice that started at differing weights. Male mice started to exhibit mortality at 1,500 mg/kg with 8/10 surviving the 1,500 mg/kg dose, 3/10 surviving the 3,000 mg/kg dose, and none surviving the 6,000 mg/kg dose of TCE until the end of the study. For females, 1 animal out of 10 died in the 750, 1,500, and 3,000 mg/kg dose groups and one surviving the 6,000 mg/kg group. In general, the magnitude of increase in TCE exposure concentration was similar to the magnitude of increase in percent liver/body weight for the 750 and 1,500 mg/kg TCE exposure groups in male B6C3F1 mice and for the 750 to 3,000 mg/kg TCE exposure groups in female mice (i.e., a 2-fold increase in TCE exposure resulted in ~2-fold increase in percent liver/body weight).

Table E-1. Mice data for 13 weeks: mean body and liver weights

Dose (mg/kg TCE)	Survival	Body weight (mean in g)		Liver weight (mean final in g)	% liver weight/BW (fold change vs. control)
		Initial	Final		
Male					
0	10/10	21	36	2.1	5.8
375	10/10	20	35	1.74	5.0 (0.86)
750	10/10	21	32	2.14	6.8 (1.17)
1,500	8/10	19	29	2.27	7.6 (1.31)
3,000	3/10	20	30	2.78	8.5 (1.46)
6,000	0/10	22	-	-	-
Female					
0	10/10	18	26	1.4	5.5
375	10/10	17	26	1.31	5.0 (0.91)
750	9/10	17	26	1.55	5.8 (1.05)
1,500	9/10	17	26	1.8	6.5 (1.18)
3,000	9/10	15	26	2.06	7.8 (1.42)
6,000	1/10	15	27	2.67	9.5 (1.73)

The descriptions of pathology in rats and mice given by this study were not very detailed. For rats only control and high dose rats were examined histologically. For mice only controls and the two highest dose groups were examined histologically. Only mean liver weights were reported with no statistical analyses provided to ascertain quantitative differences between study groups.

Pathological results were reported to reveal that 6/10 males and 6/10 female rats had pulmonary vasculitis at the highest concentration of TCE. This change was also reported to have occurred in 1/10 control male and female rats. Most of those animals were also reported to have had mild interstitial pneumonitis. The authors report that viral titers were positive during this study for Sendai virus.

In mice, liver weights (both absolute and as a percent of body weight) were reported to increase with TCE-exposure level. Liver weights were reported to have increased by more than 10% relative to controls for males receiving 750 mg/kg or more and for females receiving 1,500 mg/kg or more. The most prominent hepatic lesions detected in the mice were reported to be centrilobular necrosis, observed in 6/10 males and 1/10 females administered 6,000 mg/kg.

> Although centrilobular necrosis was not seen in either males or females administered 3000 mg/kg, 2/10 males had multifocal areas of calcifications scattered throughout their livers. These areas of calcification were considered to be evidence of earlier hepatocellular necrosis. Multifocal calcification was also seen in the liver of a single female mouse that survived the 6000 mg/kg dosage regime. One female mouse administered 3000 mg/kg also had a hepatocellular adenoma, an extremely rare lesion in female mice of this age (20 weeks).

There appeared to be consistent decrease in liver weight at the lowest dose in both female and male mice after 13 weeks of TCE exposure. Liver weight was increased at exposure concentrations in which there was not increased mortality due to TCE exposure at 13 weeks of TCE exposure.

E.2.2.12.2. ***2-year studies.*** In the 2-year phase of the NTP study, TCE was administered by corn oil gavage to groups of 50 male and 50 female F344/N rats, and B6C3F1 mice. Dosage levels were 500 and 1,000 mg/kg for rats and 1,000 mg/kg for mice. TCE was administered 5 times a week for 103 weeks and surviving animals were killed between weeks 103 and 107. The same number of animals receiving corn oil gavage served as controls. The animals were 8 weeks old at the beginning of exposure. The focus of this study was to determine if there was a carcinogenic response due to TCE exposure so there was little reporting of non-neoplastic pathology or toxicity. There was no report of liver weight at termination of the study, only body weight.

The authors reported that there was no increase in necrosis in the liver from TCE exposure in comparison to control mice. In control male mice, the incidence of hepatocellular carcinoma (tumors with markedly abnormal cytology and architecture) was reported to be 8/48 in controls, and 31/50 in TCE-exposed male mice. For females control mice hepatocellular carcinomas were reported in 2/48 of controls and 13/49 of TCE-exposed female mice. Specifically, the authors described liver pathology in mice as follows:

> Microscopically the hepatocellular adenomas were circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. The hepatocellular carcinomas had markedly abnormal cytology and architecture. Abnormalities in cytology included increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. In many instance, several or all of the abnormalities were present in different areas of the tumor. There were also variations in architecture with some of the hepatocellular carcinomas having areas of trabecular organization. Mitosis was variable in amount and location.

The authors report that the non-neoplastic lesion in male mice differing from controls was focal necrosis in 4 versus 1 animal in the dosed group (8 vs. 2%). There was no fatty metamorphosis in treated male mice versus 2 animals in control. In female mice there was focal inflammation in 29 versus 19% of animals (dosed vs. control) and no other changes. Therefore, the reported pathological results of this study did not show that the liver was showing signs of toxicity after two years of TCE exposure except for neoplasia.

For hepatocellular adenomas the incidence was reported to be "7/48 control vs. 14/50 dosed in males and 4/48 in control vs. 16/49 dosed female mice." The administration of TCE to mice was reported to cause increased incidences of hepatocellular carcinomas in males (control, 8/48; dosed, 31/50: $p = 0.001$) and in females (control 2/48; dosed 13/49; $p < 0.005$). Hepatocellular carcinomas were reported to metastasize to the lungs in five dosed male mice and one control male mouse, while none were observed in females. The incidences of hepatocellular adenomas were reported to be increased in male mice (control 7/48; dosed 14/50) and in female mice (control 4/48; dosed 16/49; $p < 0.05$). The survival of both low and high dose male rats and dosed male mice was reported to be less than that of vehicle controls with body weight decreases dose dependent. Female mice body weights were comparable to controls. The authors report adjusted rates of 20.6% for control versus 53.1% for dosed males for adenoma, 22.1% control, and 92.9% for carcinoma in males, and liver carcinoma or adenoma adjusted rates of 100%. For female mice the adjusted rates were reported to be 12.5% adenoma for control versus 55.6% for dosed, and 6.2% control carcinoma versus 43.9% dosed, with liver carcinoma or adenoma adjusted rates of 18.7% for control versus 69.7% for dosed. All of the liver results for male and female mice were reported to be statistically significant. The administration of TCE was reported to cause earlier expression of tumors as the first animals with carcinomas were 57 weeks for TCE-exposed animals and 75 weeks for control male mice.

In male rats there was no reported treatment related non-neoplastic liver lesions. In female rats a decrease in basophilic cytological change was reported to be of note in TCE treated rats (~50% in controls but ~5% in TCE treatment groups). However, the authors reported that "the results in male F344/N rats were considered equivocal for detecting a carcinogenic response because both groups receiving TCE showed significantly reduced survival compared to vehicle controls (35/70, 70%; 20/50, 40%; 16/50, 32%) and because 20% of the animals in the high-dose group were killed accidently by gavage error." Specifically 1 male control, 3 low-dose males, 10 high-dose males, 2 female controls, 5 low-dose females and 5 high-dose female rats were killed by gavage error.

E.2.2.13. *National Toxicology Program (NTP), 1988*

The studies described in the NTP (1988) TCE report were conducted "to compare the sensitivities of four strains of rats to diisopropylamine-stabilized TCE." However, the authors conclude

> that because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data, the studies are considered inadequate for either comparing or assessing TCE-induced carcinogenesis in these strains of rats. TCE (more than 99% pure, stabilized with 8ppm diisopropylamine) was administered via corn oil gavage at exposure concentrations of 0, 500 or 1000 mg/kg per day, 5 days per week, for 103 weeks to 50 male and female rats of each strain. The survival of "high-dose male Marshal rats was reduced by a large number of accidental deaths (25 animals were accidentally killed).

However, the report notes survival was decreased at both exposure levels of TCE because of mortality that occurred during the administration of the chemical. The number of animals accidently killed were reported to be: 11 male ACI rats at 500 mg/kg, 18 male ACI rats at 1,000 mg/kg, 2 vehicle control female ACI rats, 14 female ACI rats at 500 mg/kg, 12 male ACI rats at 1,000 mg/kg, 6 vehicle control male August rats, 12 male August rats at 500 mg/kg, 11 male August rats at 1,000 mg/kg, 1 vehicle control female August rats, 6 female August rats at 500 mg/kg, 13 male August rats at 1,000 mg/kg, 2 vehicle control male Marshal rats, 12 male Marshal rats at 500 mg/kg, 25 male Marshal rats at 1,000 mg/kg, 3 vehicle control female Marshal rats, 14 female Marshal rats at 500 mg/kg, 18 female Marshal rats at 1,000 mg/kg, 1 vehicle control male Osborne-Mendel rat, 6 male Osborne-Mendel rats at 500 mg/kg, 7 male

Osborne-Mendel rats at 1,000 mg/kg, 8 vehicle control female Osborne-Mendel rats, 6 female Osborne-Mendel rats at 500 mg/kg, and 6 female Osborne-Mendel rats at 1,000 mg/kg. The age of the rats "when placed on the study" were reported to differ and were for ACI rats (6.5 weeks), August rats (8 weeks), Marshal rats (7 weeks), and Osborne-Mendel rats (8 weeks). The ages of sacrifice also varied and were 17−18 weeks for the ACI and August rats, and 110−111 weeks for the Marshal rats.

Results from a 13-week study were briefly mentioned in the report. For the 13-week duration of exposure, groups of 10 male ACI and August rats were administered 0,125, 250, 500, 1,000, or 2,000 mg/kg TCE in corn oil gavage. Groups of 10 female ACI and August rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg/kg TCE. Groups of 10 male Marshal rats received 0, 268, 308, 495, 932, or 1,834 mg/kg and groups of female Marshal rats were given 0, 134, 153, 248, 466, or 918 mg/kg TCE. With the exception of 3 male August rats receiving 2,000 mg/kg TCE, all animals survived to the end of the 13-week experimental period. "The administration of the chemical for 13 weeks was not associated with histopathological changes."

In the 2-year study the report noted that there

> was no evidence of liver toxicity described as non-neoplastic changes in male ACI rats due to TCE exposure with 4% or less incidence of any lesion in control or treated animals. For female ACI rats, the incidence of fatty metamorphosis was 6% in control vehicle, 9% in low dose TCE, and 13% in high dose TCE groups. There was also a 2%, 11%, and 8% incidence of clear cell change, respectively. A 6% incidence of hepatocytomegaly was reported in vehicle control and 15% incidence in the high dose group.

All other descriptors had reported incidences of less than 4%. For August rats there was also little evidence of liver toxicity. In male August rats there was a reported incidence of 8, 4, and 10% focal necrosis in vehicle control, low dose, and high dose, respectively. Fatty metamorphosis was reported to be 8% in control, and 2 and 4% in low and high dose. All other descriptors were reported to be less than 4%. In female August rats, all descriptors of pathology were reported to have a 4% or less incidence except for hepatomegaly, which was 10% for vehicle control, 6% for the low dose and 2% for high dose TCE. For male Marshal rats there was a reported 63% incidence of inflammation, NOS in vehicle control, 12% in low dose and values not recorded at the high dose. There was a reported 6 and 14% incidence of fatty metamorphosis in control and low dose male rats. Clear cell change was 8% in vehicle with all other values 4% or less. For female Marshal rats, all values were 4% or less except for fatty metamorphosis in 6% of vehicle controls. For male Osborne-Mendel rats, there was a reported 4, 10, and 4% incidence of focal necrosis in vehicle control, low and high dose respectively. For "cytoplasmic change/NOS," there were reported incidences of 26, 32, and 27% in vehicle, low dose, and high dose animals, respectively. All other descriptors were reported to be 4% or less. In female Osborne-Mendel rats there was a reported incidence of 10% of focal necrosis at the low dose with all other descriptors reported at 4% or less.

Obviously the negative results in this bioassay are confounded by the killing of a large portion of the animals accidently by experimental error. Still, these large exposure concentrations of TCE did not seem to be causing overt liver toxicity in the rat. Organ weights were not reported in this study, which would have been hard to interpret if they had been reported because of the mortality.

E.2.2.14. *Fukuda et al., 1983*

In this 104-week bioassay designed primarily to determine a carcinogenic response, female noninbred Crj:CD-1 (ICR) mice and female Crj:CD (S-D) rats 7 weeks of age were exposed to "reagent grade" TCE at 0, 50, 150, and 450 ppm for 7 hours a day, 5 days a week. During the 2-year duration of the experiment inhalation concentrations were reported to be within 2% of target values. The numbers of animals per group were reported to be 49−50 mice and 49−51 rats at the beginning of the experiment. The impurities in the TCE were reported to be 0.128% carbon tetrachloride benzene, 0.019% epichlorohydrin and 0.019% 1,1,2-trichloroethane. After 107 weeks from commencement of the exposure, surviving animals were reported to be killed and completely necropsied. "Tumors and abnormal organs as well as other major organs were excised and prepared for examination in H&E sections." No other details of the methodologies used for pathological examination of tissues were given including what areas of the liver and number of sections examined by light microscopy.

Body weights were not given but the authors reported that "body weight changes of the mice and rats were normal with a normal range of standard deviation." It was also reported that there were no significant differences in average body weight of animals at specified times during the experiments and no significant difference in mortality between the groups of mice. The report includes a figure showing, that for the first 60 weeks of the experiment, there was a difference in cumulative mortality at the 450 ppm dose in ICR mice and the other groups. The authors reported that significantly increased mortalities in the control group of rats compared to the other dosed groups were observed at 85 weeks and after 100 weeks reflecting many deaths during the 81−85 week and 96−100 week periods for control rats. No significant comparable clinical observations were reported to be noted in each group but that major symptoms such as bloody nasal discharge (in rats), local alopecia (in mice and rats), hunching appearance (in mice) and respiratory disorders (in mice and rats) were observed in some animals mostly after 1 year.

The authors report that "the numbers of different types of tumors were counted and only malignant tumors were counted when both malignant and benign tumors were observed within one organ." They also reported that "all animals were included in the effective numbers except for a few that were killed accidently, severely autolyzed or cannibalized, and died before the first appearance of tumors among the groups." In mice the first tumors were observed at 286 days as thymic lymphoma and most of the malignant tumors appearing later were described as lymphomas or lymphatic leukemias. The incidences of mice with tumors were 37, 36, 54, and 52% in the control, 50-, 150- and 450-ppm groups, respectively, by the end of the experiment. "Tumors of the ovary, uterus, subcutaneous tissue, stomach, and liver were observed in the dose groups at low incidences (2-7%) but not in the controls." For the liver, the control, 50- and 150-ppm groups were all reported to have no liver tumors with one animal (2%) having an adenoma at the 450 ppm dose. For rats the first tumor was reported to be observed at 410 days and for the incidences of animals with tumors to be 64, 78, 66, and 63% for control, 50-ppm, 150-ppm, and 450-ppm TCE, respectively, by the end of the experiment. Most tumors were distributed in the pituitary gland and mammary gland with other tumors reported at a low incidence of 2−4% with none in the controls. For the liver there were no liver tumors in the control or 150-ppm groups but 1 animal (2%) had a cystic cholangioma in 50-ppm group and one animal (2%) had a hepatocellular carcinoma in the 450-ppm group of rats. No details concerning the pathology of the liver or organ weight changes were given by the authors, including any incidences of hepatomegaly or preneoplastic foci. On note is that in these strains, there were no background liver tumors in either strain, indicative of the relative insensitivity of these strains to hepatocarcinogenicity. However, the carcinogenic potential of TCE was reflected by a number of other tumor sites in this paradigm.

E.2.2.15. *Henschler et al., 1980*

This report focused on the potential carcinogenic response of TCE in mice (NMRI random bred), rats (WIST random bred) and hamsters (Syrian random bred) exposed to 0, 100, and 500-ppm TCE for 6 hours/day 5 days/week for 18 months. The TCE used in the experiment was reported to be pure with the exception of trace amounts of chlorinated hydrocarbons, epoxides and triethanolamines (<0.000025% w/w) and stabilized with 0.0015% triethanolamine. The number of animals in each group was 30 and the ages and initial and final body weights of the animals were not provided in the report. For the period of exposure (8 am−2 pm), animals were deprived of food and water. The exposure period was for 18 months with mice and hamsters sacrificed after 30 months and rats after 36 months. "Deceased animals" were reported to be autopsied, spleen, liver, kidneys, lungs and heart weighed, and these organs, as well as stomach, central nervous system, and tumorous tissues, examined in H&E sections.

Body weight gain was reported to be normal in all species with no noticeable differences between control and exposed groups but data were not shown. However, a "clearly dose-dependent decrease in the survival rate for both male and female mice" was reported to be statistically significant in both sexes and concentrations of TCE with no other significant differences reported in other species. The increase in mortality was more pronounced in male mice, especially after 50 weeks of exposure. Hence the opportunity for tumor development was diminished due to decreased survival in TCE treated groups. No organ weights were provided for the study due to the design, in which at considerable period of time occurred between the cessation of exposure and the sacrifice of the animals and liver weights changes due to TCE may have been diminished with time. For the 30 autopsied male mice in the control group, 1 hepatocellular adenoma and 1 hepatocellular carcinoma was reported. Whether they occurred in the same animal cannot be determined from the data presentation. In the 29 animals in the100-ppm TCE exposure group 2 hepatocellular adenomas and 1 mesenchymal liver tumor were reported but no hepatocellular carcinomas also without a determination as whether they occurred in the same animal or not. In the 30 animals autopsied in the 500–ppm-exposure group no liver tumors were reported. In female mice, of the 29 animals autopsied in the control group, 30 animals autopsied in the 100 group, and the 28 animals autopsied in the 500-ppm group, there were also no liver tumors reported.

In both the 100- and 500-ppm-exposure groups, of male mice especially, low numbers of animals studied, abbreviated TCE exposure duration, and lower numbers of animals surviving to the end of the experiment, limit the power of this study to determine a treatment-related difference in liver carcinogenicity. As discussed in Section E.2.3.2 below, the use of an abbreviated exposure regime or study duration and low numbers of animals examined limits the power of a study to detect a treatment-related response. The lack of any observed background liver tumors in the female mice and a very low background level of 2 tumors in the male mice are indicative of a low sensitivity to detect liver tumors in this paradigm, which may have occurred either through its design, or a low sensitivity of mouse strain used for this endpoint. However, the carcinogenic potential of TCE in mice was reflected by a number of other tumor sites in this paradigm.

For rats and hamsters the authors reported "no dose-related accumulation of any kind of tumor in either sex of these species." For male rats there was only 1 hepatocellular adenoma reported at 100 ppm in the 30 animals autopsied and no carcinomas. For female rats there were no liver tumors reported in control animals but, more significantly, at 100 ppm there was 1 adenoma and 1 cholangiocarcinoma reported at 100 ppm and at 500 ppm 2 cholangioadenomas. Although not statistically significant, the occurrence of this relatively rare biliary tumor was observed in both TCE dose groups in female rats. The difference in survival, as reported in mice, did not affect the power to detect a response in rats, but the low numbers of animals studied, abbreviated exposure duration and apparent low sensitivity to detect a hepatocarcinogenic response suggest a study of low power. Nevertheless, the occurrence of cholangioadenomas and 1 cholangiocarcinoma in female rats after TCE treatments is of concern, especially given the relationship in origin and proximity of the bile and liver cells and the low incidence of this tumor. For hamsters the low background rate of tumors of any kind suggests that in this paradigm, the sensitivity for detection of this tumor is relatively low.

E.2.2.16. *Maltoni et al., 1986*

The report by Maltoni et al. (1986) included a series of "systematic and integrated experiments (BT 301, 302, 303, 304, 304bis, 305, 306 bis) started in sequence, testing TCE by inhalation and by ingestion." The first experiment (BT 301) was begun in 1976 and the last in 1983 with this report representing the complete report of the findings and results of project. The focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype given and no reporting of liver weight changes induced by TCE exposure.

In experiment BT 301, TCE was administered in male and female S-D rats (13 weeks at start of experiment) via olive oil gavage at control, 50 mg/kg or 250 mg/kg exposure levels for 52 weeks (4−5 days weekly). The animals (30 male, 30 female for each dose group) were examined during their lifetime. In experiment BT 302, male and female S-D rats (13 weeks old at start of the experiment) were exposed to TCE via inhalation at 0, 100, and 600 ppm, 7 hours a day, 5 days a week, for 8 weeks. The animals (90 animals in each control group, 60 animals in each 100-ppm group, and 72 animals in each 600-ppm group) were examined during their lifetime. In experiment BT 304, male and female Sprague Dawley (S-D) rats (12 weeks old at start of the experiment) were exposed TCE via inhalation at 0, 100, 300, and 600 ppm 7 hours a day, 5 days a week, for 104 weeks. The animals (95 male, 100 female rats control groups, 90 animals in each 100-ppm group, 90 animals in each 300-ppm group, and 90 animals in each 600-ppm group) were examined during their lifetime. In experiment BT304bis, male and female S-D rats (12 weeks old at start of the experiment) were exposed to TCE via inhalation at 0, 100, 300, and 600 ppm for 7 hours a day, 5 days a week, for 104 weeks. The animals (40 male, 40 female rats control groups, 40 animals in each 100-ppm group, 40 animals in each 300-ppm group, and 40 animals in each 600-ppm group) were examined during their lifetime.

In experiment BT 303, Swiss mice (11 weeks old at the start of the experiment) were exposed to TCE via inhalation in for 8 weeks using the same exposure concentrations as for experiment BT 302. The animals (100 animals in each control group, 60 animals in the 100-ppm-exposed group, and 72 animals in each 600-ppm group) were examined during their lifetime. In experiment BT 305, Swiss mice (11 weeks old at the start of the experiment) were exposed to TCE via inhalation in for 78 weeks, 7 hours a day, 5 days a week. The animals (90 animals in each control group, 90 animals in the 100-ppm-exposed group, 90 animals in the 300-ppm group, and 90 animals in each 600-ppm group) were examined during their lifetime. In experiment BT 306, B6C3F1 mice (from NCI source) (12 weeks old at the start of the experiment) were exposed to TCE via inhalation in for 78 weeks, 7 hours a day, 5 days a week. The animals (90 animals in each control group, 90 animals in the 100-ppm-exposed group, 90 animals in the 300-ppm group, and 90 animals in each 600-ppm group) were examined during their lifetime. In experiment BT 306bis B6C3F1 mice (from Charles River Laboratory as source) (12 weeks old at the start of the experiment) were exposed to TCE via inhalation for 78 weeks, 7 hours a day, 5 days a week. The animals (90 animals in each control group, 90 animals in the 100-ppm-exposed group, 90 animals in the 300-ppm group, and 90 animals in each 600-ppm group) were examined during their lifetime.

In all experiments, TCE was supplied tested and reported by the authors of the study to be was highly purified and epoxide free with butyl-hydroxy-toluene at 20 ppm used as a stabilizer. Extra virgin olive oil was used as the carrier for ingestion experiments and was reported to be free of pesticides. The authors describe the treatment of the animals and running of the facility in detail and report that:

> Animal rooms were cleaned every day and room temperature varied from 19 degrees to 22 degrees and was checked 3 times daily. Bedding was changed every two days and cages changes and washed once weekly. The animals were handled very gently and, therefore, were neither aggressive nor nervous. Concentrations of TCE were checked by continuous gas-chromatographic monitoring. Treatment was performed by the same team. In particular, the same person carried out the gavage of the same animals. This is important, since animals become accustomed to the same operators. The inhalation chambers were maintained at 23 ± 2 degrees C and 50 ± 10% relative humidity. Ingestion from Monday to Friday was usually performed early in the morning. The status and behavior of the animals were examined at least three times daily and recorded. Every two weeks the animals were submitted to an examination for the detection of the gross changes, which were registered in the experimental records. The animals which were found moribund at the periodical daily inspection were isolated in order to avoid cannibalism. The animals were weight every two weeks during treatment and then every eight weeks. Animals were kept under

> observation until spontaneous death. A complete necropsy was performed. Histological specimens were fixed in 70% ethyl alcohol. A higher number of samples was taken when particular pathological lesions were seen. All slides were screened by a junior pathologist and then reviewed by a senior pathologist. The senior pathologist was the same throughout the entire project. Analysis of variance was used for statistical evaluation of body weights. Results are expressed as means and standard deviations. Survival time is evaluated using the Kruskal-Wallis test. For different survival rates between groups, the incidence of lesions is evaluated by using the Log rank test. Non-neoplastic, preneoplastic, and neoplastic lesions were evaluated using the Chi-square of Fisher' exact test. The effect of different doses was evaluated using the Cochran-Armitage test for linear trends in proportions and frequencies.

The authors state that: "Although the BT project on TCE was started in 1976 and most of the experiments were performed from the beginning of 1979, the methodological protocol adopted substantially met the requirements of the Good Laboratory Practices Act." Finally, it was reported that "the experiments ran smoothly with no accidents in relation to the conduct of the experiment and the health of the animals, apart from an excess in mortality in the male B6C3F1 mice of the experiment BT 306, due to aggressiveness and fighting among the animals." This is in contrast to the description of the gavage studies conducted by NTP (1990, 1988) in which gavage error resulted in significant loss of experimental animals. Questions have been raised about the findings, experimental conditions, and experimental paradigm of the European Ramazzini Foundation (ERF) from which the Maltoni et al. (1986) experiments were conducted (EFSA, 2006). However, these concerns were addressed by Caldwell et al. (2008a), who concluded that the ERF bioassay program produced credible results that were generally consistent with those of NTP

In regards to effects of TCE exposure on survival,

> a nonsignificant excess in mortality correlated to TCE treatment was observed only in female rats (treated by ingestion with the compound) and in male B6C3F1 mice. In B6C3F1 mice of the experiment BT 306 bis, the excess in mortality in treated animals was higher ($p < 0.05$ after 40 weeks) but was not dose correlated. No excess in mortality was observed in the other experiments.

The authors reported that "no definite effect of TCE on body weight was observed in any of the experiments, apart from experiment BT 306 bis, in which a slight nondose correlated decrease was found in exposed animals."

In mice, "hepatoma" was the term used by the authors of these studies to describe all malignant tumors of hepatic cells, of different subhistotypes, and of various degrees of malignancy. The authors reported that the hepatomas induced by exposure to TCE

> may be unique or multiple, and have different sizes (usually detected grossly at necropsy). Under microscopic examination these tumors proved to be of the usual type observed in Swiss and B6C3F1 mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens. They frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. The hepatomas may produce distant metastases, more frequently in the lungs.

In regard to the induction of "hepatomas" by TCE exposure, the authors report that in Swiss mice exposed to TCE by inhalation for 8 weeks (BT303), the percentage of animals with hepatomas was 1.0% in male mice and 1.0% in female mice in the control group (*n* = 100 for each gender). For animals exposed to 100 ppm TCE, the percentage in female mice was 1.7% and male mice 5.0% (*n* = 60 for each gender). For animals exposed to 600 ppm TCE, the percentage in female mice was 0% and in male mice 5.5% (*n* = 72 for each gender). The relatively larger number of animals used in this bioassay, in comparison to NTP standard assays, allows for a greater power to detect a response. It is also apparent from these results that Swiss mice in this experimental paradigm are a "less sensitive" strain in regard to spontaneous liver cancer induction over the lifetime of the animals. These results suggest that 8 weeks of TCE exposure via inhalation at 100 ppm or 600 ppm may have been associated with a small increase in liver tumors in male mice in comparison to concurrent controls.

In Swiss mice exposed to TCE via inhalation for 78 weeks (BT 305), the percentage of animals with hepatomas was reported to be 4.4% in male mice and 0% in female mice in the control group (*n* = 90 for each gender). For animals exposed to 100 ppm TCE, the percentage in female mice was reported to be 0% and male mice 2.2% (*n* = 90 for each gender). For animals exposed to 300 ppm TCE, the percentage in female mice was reported to be 0% and in male mice 8.9% (*n* = 90 for each gender). For animals exposed to 600 ppm TCE, the percentage in female mice was reported to be 1.1% and in male mice 14.4%. As with experiment BT303, there is a consistency in the relatively low background level of hepatomas reported for Swiss mice in this paradigm. After 78 weeks of exposure there appears to be a dose-related increase in hepatomas in male but not female Swiss mice via inhalation exposure.

In B6C3F1 mice exposed to TCE by inhalation for 78 weeks (BT306) the percentage of animals with hepatomas was reported to be 1.1% in male mice and 3.3% in female mice in the control group (*n* = 90 for each gender). For animals exposed to 100 ppm TCE, the percentage in female mice was reported to be 4.4% and in male mice 1.1% (*n* = 90 for each gender). For animals exposed to 300 ppm TCE, the percentage in female mice was reported to be 3.3% and in male mice 4.4% (*n* = 90 for each gender). For animals exposed to 600 ppm TCE, the percentage in female mice was reported to be 10.0% and in male mice 6.7%. This was the experimental group with excess mortality in the male group due to fighting. The excess mortality could have affected the results. The authors do report that there was a difference in the percentage of males bearing benign and malignant tumors that was due to early mortality among males in experiment BT306. It is unexpected for the liver cancer incidence to be less in male mice than female mice and not consistent with the results reported for the Swiss mice.

In B6C3F1 male mice exposed to TCE via inhalation (BT 306 bis) the percentage of animals with hepatomas was reported to be 18.9% in male mice in the control group (*n* = 90). For animals exposed to 100 ppm TCE, the percentage in male mice was reported to be 21.1% (*n* = 90). For animals exposed to 300 ppm TCE, the percentage in male mice was reported to be 30.0% (*n* = 90). For animals exposed to 600 ppm TCE, the percentage in male mice was reported to be 23.3%. This experiment did not examine female mice. The authors do report a decrease in survival in mice from this experiment that could have affected results. It is apparent from the BT 306 and BT 306 bis experiments that the background level of liver cancer was significantly different in male mice, although they were supposed to be of the same strain. The finding of differences in response in animals of the same strain but from differing sources has also been reported in other studies for other endpoints (see Section E.3.1.2, below).

The authors reported 4 liver angiosarcomas: 1 in an untreated male rat (BT 304); 1 in a male and 1 in a female rat exposed to 600 ppm TCE for 8 weeks (experiment BT302); and 1 in a female rat exposed to 600 ppm TCE for 104 weeks (BT 304). The authors conclude that

> the tumors observed in the treated animals cannot be considered to be correlated to TCE treatment, but are spontaneously arising. These findings are underlined because of the extreme rarity of this tumor in control Sprague Dawley rats, untreated or treated with vehicle materials. The morphology of these tumors is of the liver angiosarcoma type produced by vinyl chloride in this strain of rats.

In rats treated for 104 weeks, TCE was reported to not affect the percentages of animals bearing benign and malignant tumor and of animals bearing malignant tumors. Moreover, it did not affect the number of total malignant tumors per 100 animals. This study did not report a treatment related increase in liver cancer in rats. The report only explicitly described positive findings so it is assumed that there were no increases in "hepatomas" in rat liver associated with TCE treatment. The authors concluded that "under the tested experimental conditions, the evidence of TCE (without epoxide stabilizer) carcinogenicity, gives the result of TCE treatment-related hepatomas in male Swiss and B6C3F1 mice. A borderline increased frequency of hepatomas was also seen after 8 weeks of exposure in male Swiss mice." Thus, the increase in liver tumors in both strains of mice exposed to TCE via inhalation reported in this study is consistent with the gavage results from the NTP (1990) study in B6C3F1 mice, where male mice had a higher background level and greater response from TCE exposure than females.

E.2.2.17. *Maltoni et al., 1988*

This report was an abbreviated description of an earlier study (Maltoni et al., 1986) focusing on the identification of a carcinogenic response in rats and mice by chronic TCE exposure.

E.2.2.18. *Van Duuren et al., 1979*

This study exposed male and female noninbred HA:ICR Swiss mice at 6−8 weeks of age to distilled TCE with no further descriptions of purity. Gavage feeding of TCE was once weekly in 0.1 mL trioctanoin. Neither initial nor final body weights were reported by the authors. The authors reported that, at the termination of the experiments or at death, animals were completely autopsied with specimens of all abnormal-appearing tissues and organs excised for histopathologic diagnosis. Tissues from the stomachs, livers, and kidneys were reported to be taken routinely for the intragastric feeding experiments. Tissues were reported to be stained for H&E for pathologic examination, but no further description of the lobe(s) of the liver examined or the sections examined was provided by the authors. Results were as only reported the no of mice with forestomach tumors 0.5 mg/mouse of TCE treatment given once a week in 0.1 mL trioctanoin. Mouse body weights were not given so the dose in mg/kg for the mice cannot be ascertained. The protocol used in this experiment kept the mg/mouse constant with a 1 week dosing schedule so that as the mice increased weight with age, the dose as a function of body weight was decreased. The days on test were reported to be 622 for 30 male and female mice. 2 male and 1 female mice were reported as having forestomach tumors. For 30 mice treated with trioctanoin alone the number of forestomach tumors was reported to be zero. For mice with no TCE treatment, 5 of 100 male mice were reported to have forestomach tumors and of 8 of 60 female mice were reported to have forestomach tumors for 636 and 649 days on test. No results for liver were presented by the authors by the intragastric route of administration including background rates of the incidences of liver tumors or treatment results. The authors note that except for repeated skin applications of certain chemicals, no significant difference between the incidence of distant tumors in treated animals compared with no-treatment and vehicle control groups was noted. Given the uncertainties in regard to dose, the once-a week dosing regime, the low number of animals tested with resulting low power, and the lack of reporting of experimental results, the ability to use the results from this experiment in regard to TCE carcinogenicity is very limited.

E.2.2.19. *National Cancer Institute (NCI), 1976*

This bioassay was "initiated in 1972 according to the methods used and widely accepted at that time" with the design of carcinogenesis bioassays having "evolved since then in some respects and several improvements" having been developed. The most notable changes reported in the foreward of the report are changes "pertaining to preliminary toxicity studies, numbers of controls used, and extent of pathological examination." Industrial grade TCE was tested (99% TCE, 0.19% 1,2,-epoxybutane, 0.04%v ethyl acetate, 0.09% epichlorhydrin, 0.02% *N*-methyl pyrrole, and 0.03% diisobutylene) with rats and mice exposed via gavage in corn oil 5 times/week for 78 weeks using 50 animals per group at 2 doses with both sexes of Osborne-Mendel rats and B6C3F1 mice. However, for control groups only 20 of each sex and species were used. Rats were killed after 110 weeks and mice after 90 weeks. Rats and mice were initially 48 and 35 days of age, respectively, at the start of the experiment with control and treated animals born within 6 days of each other. Initial weight ranges were reported as ranges for treated and control animals of 168−229 g for male rats, 130−170 g for female rats, 11−22 g for male mice, and 11−18 g for female mice. Animals were reported to be "randomly assigned to treatment groups so that initially the average weight in each group was approximately the same." Mice treated with TCE were reported to be

> maintained in a room housing other mice being treated with one of the following 17 compounds: 1,1,2-2-tetrachloroethane, chloroform, 3-chloropropene, chloropicrin, 1,2-dibromochloropropane, 1,2, dibromoethane, ethylene dichloride, 1,1-diochloroethane, 3-sulfolene, idoform, methyl chloroform, 1,1,2-trichloroethane, tetrachloroethylene, hexachloroethane, carbon disulfide, trichlorofluoromethane, and carbon tetrachloride. Nine groups of vehicle controls and 9 groups of untreated controls were also housed in this same room.

The authors note that

> TCE-treated rats and their controls were maintained in a room housing other rats being treated with one of the following compounds: dibromochloropropane, ethylene dichloride, 1,1-dichloroethane, and carbon disulfide. Four groups of vehicle-treated controls were in the same room." Thus, there was the potential of co-exposure to a number of other chemicals, especially for the mice, resulting from exhalation in treated animals housed in the same room, including the control

groups, as noted by the authors. The authors also noted that "samples of ambient air were not tested for presence of volatile materials" but state that "although the room arrangement is not desirable as is stated in the Guidelines for Carcinogen Bioassay in Small Rodents, there is not evidence the results would have been different with a single compound in a room.

The initial doses of TCE for rats were reported to be 1,300 and 650 mg/kg. However, these levels were changed based on survival and body weight data "so that the time-weighted average doses were 549 and 1097 mg/kg for both male and female rats." For mice, the initial doses were reported to be 1,000 and 2,000 mg/kg for males and 700 and 1,400 mg/kg for females. The "doses were increased so that the time weighted average doses were 1169 mg/kg and 2339 mg/kg for male mice and 869 and 1739 mg/kg for female mice." The authors reported that signs of toxicity, including reduction in weight, were evident in treated rats, which, along with increased mortality, "necessitated a reduction in doses during the test." In contrast "very little evidence of toxicity was seen in mice, so doses were increased slightly during the study." Doses were "changed for the rats after 7 and 16 weeks of treatment, and for the mice after 12 weeks." At 7 weeks of age, male and female rats were dosed with 650mg/kg TCE, at 14 weeks they were dosed with 750 mg/kg TCE, and at 23 weeks of age 500 mg/kg TCE. For the high exposure level, the exposure concentrations were 1,300, 1,500, and 1,000 mg/kg TCE, respectively, for the same changes in dosing concentration. For rats the percentage of TCE in corn oil remained constant at 60%. For female mice, the TCE exposure at the beginning of dosing was 700 mg/kg TCE (10% in corn oil) at 5 weeks of age for the "lower dose" level. The dose was increased to 900 mg/kg day (18% in corn oil) at 17 weeks of age and maintained until 83 weeks of age. For male mice, the TCE exposure at the beginning of dosing was 1,000 mg/kg TCE (15% in corn oil) at 5 weeks of age for the "lower dose" level. At 11 weeks, the level of TCE remained the same but the percentage of TCE in corn oil was reduced to 10%. The dose was increased to 1,200 mg/kg day at 17 weeks of age (24% in corn oil) and maintained until 83 weeks of age. For the "higher dose," the TCE exposure at the beginning of dosing was 1,400 mg/kg TCE (10% in corn oil) at 5 weeks of age in female mice. At 11 weeks of age the exposure level of TCE was kept the same but the percentage of TCE in corn oil increased to 20%. By 17 weeks of age the exposure concentration of TCE in corn oil was increased to 1,800 mg/kg (18% in corn oil) in female mice. For the "higher dose" in male mice, the TCE exposure at the beginning of dosing was 2,000 mg/kg (15% in corn oil) which was maintained at 11 weeks in regard to TCE administered but the percent of TCE corn oil was increased to 20%. For male mice the exposure concentration was increased to 2,400 mg/kg (24% in corn oil). For all of the mice treatment continued on a 5 days/week schedule of oral gavage dosing throughout the timecourse of treatment (78 weeks of treatment). Thus, not only did the total dose administered to the animals change, but the volumes of vehicle in which TCE was administered changed throughout the experiment.

The authors stated that at 37 weeks of age, "To help assure survival until planned termination the dosing schedule was changed for rats to a cycle of 1 week of no treatment followed by 4 weeks of treatment." for male and female rats. Thus, the duration of exposure in rats was also changed. All lobes of the liver were reported to be taken including the free margin of each lobe with any nodule or mass represented in a block 10 × 5 × 3 mm cut from the liver and fixed in a marked capsule.

Body weights (mean ± SD) were reported to be 193 ± 15.0 g (n = 20), 193 ± 15.8 g (n = 50), and 195 ± 16.7 g (n = 50) for control, low, and high dose male rats at initiation of the experiment. By 1 year of exposure (50 weeks), 20/20 control male rats were still alive to be weighed, 42/50 of the low dose rats were alive and 34/50 of high dose rats were still alive. The body weights of those remaining were decreased by 6.2 and 17% in the low and high dose animals in comparison with the controls. For female rats, the mean body weights were reported to be 146 ± 11.4 g (n = 20), 144 ± 11.0 g (n = 50), and 144 ± 9.5 g (n = 50) for control, low, and high dose female rats at initiation of the experiment. By 1 year of exposure (50 weeks), 17/20 control female rats were still alive, 28/50 low dose and 39/50 of the high dose rats were alive. The body weights of those remaining were decreased by 25 and 30% in the low and high dose animals in comparison with the controls. For male mice the initial body weights were 17 ± 0.5 g (n = 20), 17 ± 2.0 g (n = 50), and 17 ± 1.1 g (n = 50) for control, low and high doses. By 1 year of exposure (50 weeks), 18/20 control male mice were still alive, 47/50 or the low dose, and 34/50 of the high-dose groups were still alive. The body weights of those remaining were unchanged in comparison to controls. For female mice the initial body weights were 14 ± 0.0 g (n = 20), 14 ± 0.6 g (n = 50), and 14 ± 0.7 g (n = 50) for control, low and high doses. By 1 year of exposure (50 weeks), 18/20 control male mice were still alive, 45/50 or the low dose, and 41/50 of the high-dose groups were still alive. The body weights of those remaining were unchanged in comparison to controls.

A high proportion of rats were reported to die during the experiment with 17/20 control, 42/50 low dose, and 47/50 high dose animals dying prior to scheduled termination. For female rats, 12/20 control, 35/48 low dose, and 37/50 high dose animals were reported to die before scheduled termination with two low dose females reported to be missing and not counted in the denominator for that group. The authors reported that earlier death was associated with higher TCE dose. A decrease in the percentage of tumor-bearing animals was reported to be lower in treated animals and attributed by the authors to be likely related to the decrease in their survival.

A high percentage of respiratory disease was reported to be observed among the rats without any apparent difference in the type, severity, or morbidity as to sex or group. The authors reported that "no significant toxic hepatic changes were observed" but no other details regarding results in the liver of rats. Carbon tetrachloride was administered to rats as a positive control. A low incidence of both hepatocellular carcinoma and neoplastic nodule was reported to be found in both colony controls (1/99 hepatocellular carcinoma and 0/99 neoplastic nodule in male rats and 0/98 hepatocellular carcinoma and 2/98 neoplastic nodules in female rats) and carbon-tetrachloride-treated rats. Hepatic adenomas were included in the description of neoplastic nodules in this study with the diagnosis of hepatocellular carcinoma to be "based on the presence of less organized architecture and more variability in the cells comprising the neoplasms."

The authors reported that "increased mortality in treated male mice appears to be related to the presence of liver tumors." For mice both male and female mice the incidences of hepatocellular carcinoma were reported to be high from TCE treatment with 1/20 in age matched controls, 26/50 in low dose and 31/48 in high dose males. Colony controls for male mice were reported to be 5/77 for vehicle and 5/70 for untreated mice. For females mice hepatocellular carcinomas were reported to be observed in 0/20 age matched controls, 4/50 low dose, and 11/47 high-dose female mice. Colony controls for female mice were reported to be 1/80 for vehicle and 2/75 for untreated mice. In male mice, hepatocellular carcinomas were reported to be observed early in the study with the first seen at 27 weeks. Hepatocellular carcinomas were not observed so early in low dose male or female mice.

The diagnosis of hepatocellular carcinoma was reported to be based on histologic appearance and the presence of metastasis especially to the lung with not other lesions significantly elevated in treated mice. The tumors were reported to be

> varied from those composed of well differentiated hepatocytes in a relatively uniform trabecular arrangement to rather anaplastic lesions in which mitotic figures occurred in cells which varied greatly in size and tinctorial characteristics. Many of the tumors were characterized by the formation of relatively discrete areas of highly anaplastic cells within the tumor proper which were, in turn, surrounded by relatively well differentiated neoplastic cells. In general, various arrangements of the hepatocellular carcinoma occurred, as described in the literature, including those with an orderly cord-like arrangement of neoplastic cells, those with a pseudoglandular pattern resembling adenocarcinoma, and those composed of sheets of highly anaplastic cells with minimal cord or gland-like arrangement. Multiple metaplastic lesions were observed in the lung, including several neoplasms which were differentiated and relative benign in appearance." The authors noted that almost all mice treated with carbon tetrachloride exhibited liver tumors and that the "neoplasms occurring in treated [sic carbon tetrachloride

treated] mice were similar in appearance to those noted in the trichloroethylene-treated mice.

Thus, phenotypically this study reported that the liver tumors induced in mice by TCE were heterogeneous and typical of those arising after carbon tetrachloride administration. The descriptions of liver tumors in this study and the tendency of metastasis to the lung are similar to the descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation.

In terms of noncancer pathology of the liver, 1 control male rat was reported to display fatty metamorphosis of the liver at 102 weeks. However, for the low dose, 3 male rats were reported to display fatty metamorphosis (90, 110, and 110 weeks), 2 rats to display cystic inflammation (76, 110 weeks), and one rat to display general inflammation (110 weeks). At the high dose, 6 rats were reported to display fatty metamorphosis (12, 35, 49, 52, 52, and 58 weeks), 1 rat was reported to display cytomegaly (42 weeks), 2 rats were reported to display centrilobular degeneration (53 and 58 weeks), 1 rats to display diffuse inflammation (62 weeks), 1 rat to display congestion (Week 12), and 5 rats to display angiectasis or abnormally enlarged blood vessels which can be manifested by hyperproliferation of endothelial cells and dilatation of sinusoidal spaces (35, 42, 52, 54, and 65 weeks). One control female rat was reported o display fatty metamorphosis of the liver at 110 weeks, and one control female rats to display "inflammation" of the liver at 110 weeks. Of the TCE dosed female rats, only 1 high dose female rat displayed fatty metaphorphosis at Week 96. Thus, for male rats, there was liver pathology present in some rats due to TCE exposure examined from 12 weeks to a year at their time of their premature death. For mice the liver pathology was dominated by the presence of hepatocellular carcinoma with additional hyperplasia noted in 2 mice of the high dose male and female groups and 1 or less mouse exhibiting hyperplasia in the control or low-dose groups.

The authors note that "while the absence of a similar effect in rats appears most likely attributable to a difference in sensitivity between the Osborne-Mendel rat and B6C3F1 mouse, the early mortality of rats due to toxicity must also be considered." The conclude that "the test in rats is inconclusive: large numbers of rats died prior to planned termination; in addition, the response of this rat strain to the hepatocarcinogenicity of the positive control compound, carbon tetrachloride, appeared relatively low." Finally, the authors note that "while the results obtained in the present bioassay could possibly have been influenced by an impurity in the TCE used, the extremely low amounts of impurities found make this improbable."

E.2.2.20. *Herren-Freund et al., 1987*

This study was gave results primarily in initiated male B6C3 F1 mice that were also exposed to TCE metabolites in drinking water for 61 weeks. However, in Table 1 of the report, results were given for mice that received no initiator but were given 40 mg/L TCE or 2 g/L NaCl as control. The mice were reported to be 28 days of age when placed on drinking water containing TCE. The authors reported that concentrations of TCE fell by about ½ at the 40 mg/L dose of TCE during the twice a week change in drinking water solution. For control animals (n = 22) body weight at termination was reported to be 32.93 ± 0.54 g, and liver weight was 1.80 ± 0.05 g, percent liver/body weight was 5.47% ± 0.16%. For TCE treated animals (n = 32), body weight at termination was reported to be 35.23 ± 0.66 g, and liver weight was 1.97 ± 0.10 g, percent liver/body weight was 5.57% ± 0.24%. Thus, hepatomegaly was not reported for this paradigm at this time of exposure. The study reported that for 22 control animals, the prevalence of adenomas was 2/22 animals (or 9%) with the mean number of adenomas per animal to be 0.09 ± 0.06 (SEM). The prevalence of carcinomas in the control group was reported to be 0/22. For 32 animals exposed to 40 mg/L TCE, the prevalence of adenomas was 3/32 animals (or 9%) with the mean number of adenomas per animal to be 0.19 ± 0.12 (SEM). The prevalence of animals with hepatocellular carcinomas was 3/32 animals (or 9%) with the mean number of hepatocellular carcinomas to be 0.10 ± 0.05 (SEM). Thus, similar to the acute study of Tucker et al. (1982), significant loss of TCE is a limitation for trying to evaluate TCE hazard in drinking water. However, despite difficulties in establishing accurately the dose received, an increase in adenomas per animal and an increase in the number of animals with hepatocellular carcinomas were reported to be associated with TCE exposure after 61 weeks of exposure. Also of note is that the increase in tumors was reported without significant increases in hepatomegaly at the end of exposure. The authors did not report these increases in tumors as being significant but did not do a statistical test between TCE exposed animals without initiation and control animals without initiation. The low numbers of animal tested limits the statistical power to make such a determination. However, for carcinomas, there was none reported in controls but 9% of TCE-treated mice had hepatocellular carcinomas.

E.2.2.21. *Anna et al., 1994*

The report focused on presenting incidence of cancer induction after exposure to TCE or its metabolites and included a description of results for male B6C3F1 mice (8 weeks old at the beginning of treatment) receiving 800 mg/kg/d TCE via gavage in corn oil, 5 days/week for 76 weeks. There was very limited reporting of results other than tumor incidence. There was no reporting of liver weights at termination of the experiment. Although the methods section of the report gives 800 mg/kg/d as the exposure level, Table 1 in the results section reports that TCE was administered at 1,700 mg/kg/d. This could be a typographical error in the table as a transposition with the dose of "perc" administered to other animals in the same study. The methods section of the report states that the authors based their dose in mice that used in the 1990 NTP study. The NTP study only used a1,000 mg/kg/d in mice suggesting that the table is mislabeled and suggests that the actual dose is 800 mg/kg/d in the Anna et al. (1994) study. All treated mice were reported to be alive after 76 weeks of treatment. For control animals, 10 animals exposed to corn oil, and 10 untreated controls were killed in a 9-day period. The remaining controls were killed at 96, 103, 134 weeks of treatment. Therefore, the control group (all) contains a mixed group of animals that were sacrificed from 76−134 weeks and were not comparable to the animals sacrificed at 76 weeks. At 76 weeks 3 of 10 the untreated and two of the 10 corn oil treated controls were reported to have one small hepatocellular adenoma. None of the controls examined at 76 weeks were reported to have any observed hepatocellular carcinomas. The authors reported no cytotoxicity for TCE, corn oil, and untreated control group. At 76 weeks, 75 mice treated with 800 mg/kg/d TCE were reported to have a prevalence of 50/75 animals having adenomas with the mean number of adenomas per animal to be 1.27 ± 0.14 (SEM). The prevalence of carcinomas in these same animals was reported to be 30/70 with the mean number of hepatocellular carcinomas per animal to be 0.57 ± 0.10 (SEM). Although not comparable in terms of time till tumor observation, Corn oil control animals examined at much later time points did not have as great a tumor response as did those exposed to TCE. At 76−134 weeks 32 mice treated with corn oil were reported to have a prevalence of 4/32 animals having adenomas with the mean number of adenomas per animal to be 0.13 ± 0.06 (SEM). The prevalence of carcinomas in these same animals was reported to be 4/32 with the mean number of hepatocellular carcinomas per animal to be 0.12 ± 0.06 (SEM). Despite only examining one exposure level of TCE and the limited reporting of findings other than incidence data, this study also reported that TCE exposure in male B6C3F1 mice to be associated with increased induction of adenomas and hepatocellular carcinoma, without concurrent cytotoxicity.

In terms of liver tumor phenotype, Anna et al. reported the percent of H-ras codon 61 mutations in tumors from concurrent control animals (water and corn oil treatment groups combined) examined in their study, historical controls in B6C3 F1mice, and in tumors from TCE or DCA (0.5% in drinking water) treated animals. From their concurrent controls they reported that H-ras codon 61 mutations in 17% (n = 6) of adenomas and 100% (n = 5) of carcinomas. For historical controls (published and unpublished) they reported mutations in 73% (n = 33) of adenomas and mutations in 70% (n = 30) of carcinomas. For tumors from TCE treated animals they reported mutations in 35% (n = 40) of adenomas and 69% (n = 36) of carcinomas, while for

DCA treated animals they reported mutations in 54% (*n* = 24) of adenomas and in 68% (*n* = 40) of carcinomas. The authors reported that "in this study, the H-ras codon 61 mutation frequency was not statistically different in liver tumors from dichloroacetic acid and trichloroethylene-treated mice and combined controls (62%, 51% and 69%, respectively)." In regard to mutation spectra in H-ras oncogenes detected B6C3F1 mouse liver "tumors," the authors reported combined results for concurrent and historical controls of 58% AAA, 27% CGA, and 14% CTA substitutions for CAA at Codon 61 out of 58 mutations. For TCE "tumors" the substitution pattern was reported to be 29% AAA, 24% CGA, and 40% CTA substitutions for CAA at Codon 61 out of 39 mutations and for DCA 28% AAA, 35% CGA, and 38% CTA substitutions for CAA at Codon 61 out of 40 mutations.

E.2.2.22. *Bull et al., 2002*

This study primarily presented results from exposures to TCE, DCA, TCA and combinations of DCA and TCA after 52 weeks of exposure with some animals examined at 87 weeks. It only examined and described results for liver. In a third experiment, 1,000 mg/kg TCE was administered once daily 7 days a week for 79 weeks in 5% alkamuls in distilled water to 40 B6C3F1 male mice (6 weeks old at the beginning of the experiment). At the time of euthanasia, the livers were removed, tumors identified, and the tissues section of for examination by a pathologist and immunostaining. Liver weights were not reported. For the TCE gavage experiment there were 6 gavage-associated deaths during the course of this experiment among a total of 10 animals that died with TCE treatment. No animals were lost in the control group. The limitations of this experiment were discussed in Caldwell et al. (2008b). Specifically, for the DCA and TCA exposed animals, the experiment was limited by low statistical power, a relatively short duration of exposure, and uncertainty in reports of lesion prevalence and multiplicity due to inappropriate lesions grouping (i.e., grouping of hyperplastic nodules, adenomas, and carcinomas together as "tumors"), and incomplete histopathololology determinations (i.e., random selection of gross lesions for histopathology examination). For the reported TCE results, Bull et al. (2002) reported a high prevalence (23/36 B6C3F1 male mice) of adenomas and hepatocellular carcinoma (7/36) and gave results of an examination of approximately half of the lesions induced by TCE exposure. Tumor incidence data were provided for only 15 control mice and reported as 2/15 (13%) having adenomas and 1/15 (7%) carcinomas. Thus, this study presents results that are consistent with other studies of chronic exposure that show TCE induction of hepatocellular carcinoma in male B6C3F1 mice.

For determinations of immunoreactivity to c-Jun as a marker of differences in "tumor" phenotype, Bull et al. (2002) did include all lesions in most of their treatment groups, decreasing the uncertainty of his findings. The exceptions were the absence of control lesions and inclusion of only 16/27 and 38/72 lesions for 0.5 g/L DCA + 0.05 g/L TCA and 1 g/kg/day TCE exposure groups, respectively. Immunoreactivity results were reported for the group of hyperplastic nodules, adenomas, and carcinomas. Thus, changes in c-Jun expression between the differing types of lesions were not determined. Bull et al. (2002) reported lesion reactivity to c-Jun antibody to be dependent on the proportion of the DCA and TCA administered after 52 weeks of exposure. Given alone, DCA produced lesions in mouse liver for which approximately half displayed a diffuse immunoreactivity to a c-Jun antibody, half did not, and none exhibited a mixture of the two. After TCA exposure alone, no lesions were reported to be stained with this antibody. When given in various combinations, DCA and TCA coexposure induced a few lesions that were only c-Jun+, many that were only c-Jun−, and a number with a mixed phenotype whose frequency increased with the dose of DCA. For TCE exposure of 79 weeks, TCE-induced lesions also had a mixture of phenotypes (42% c-Jun+, 34% c-Jun−, and 24% mixed) and were most consistent with those resulting from DCA and TCA coexposure but not either metabolite alone.

Mutation frequency spectra for the H-ras codon 61 in mouse liver "tumors" induced by TCE (n = 37 tumors examined) were reported to be significantly different than that for TCA (n = 41 tumors examined), with DCA-treated mice tumors giving an intermediate result (n = 64 tumors examined). In this experiment, TCA-induced "tumors" were reported to have more mutations in codon 61(44%) than those from TCE (21%) and DCA (33%). This frequency of mutation in the H-ras codon 61 for TCA is the opposite pattern as that observed for a number of peroxisome proliferators in which the mutation spectra in tumors has been reported to be much lower than spontaneously arising tumors (see Section E.3.4.1.5). Bull et al. (2002) noted that the mutation frequency for all TCE,TCA or DCA was lower in this experiment than for spontaneous tumors reported in other studies (they had too few spontaneous tumors to analyze in this study), but that this study utilized lower doses and was of shorter duration than that of Ferreira-Gonzalez et al. (1995). These are additional concerns along with the effects of lesion grouping in which a lower stage of progression is group with more advanced stages. In a limited subset of tumor that were both sequenced and characterized histologically, only 8 of 34 (24%) TCE-induced adenomas but 9/15 (60%) of TCE-induced carcinomas had mutated H-ras at codon 61, which the authors suggest is evidence that this mutation is a late event.

The issues involving identification of MOA through tumor phenotype analysis are discussed in detail below for the more general case of liver cancer as well as for specific hypothesized MOAs (see Sections E.3.1.4, E.3.1.8, E.3.2.1, and E.3.4.1.5). In an earlier paper, Bull (2000) suggested that "the report by Anna et al (1994) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-ras oncogene than those observed in spontaneous tumors of control mice." Bull (2000) stated that "results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism" but went on to suggest that it is not possible to *a priori* rule out a role for selection in this process and that differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver tumors. Bull (2000) noted that data from Anna et al. (1994), Ferreira-Gonzalez et al. (1995), and Maronpot et al. (1995) indicated that mutation frequency in DCA-induced tumors did not differ significantly from that observed in spontaneous tumors, that the mutation spectra found in DCA-induced tumors has a striking similarity to that observed in TCE-induced tumors, and DCA-induced tumors were significantly different than that of TCA-induced liver tumors. What is clear from these observations is the phenotype of TCE-induced tumors appears to be more like DCA-induced tumors (which are consistent with spontaneous tumors), or those resulting from a coexposure to both DCA and TCA, than from those induced by TCA. More importantly, these data suggest that using measures other than dysplasticity and tincture indicate that mouse liver tumors induced by TCE are heterogeneous in phenotype. The descriptions of tumors in mice reported by the NTP and Maltoni et al studies are also consistent with phenotypic heterogeneity as well as consistency with spontaneous tumor morphology.

E.2.3. Mode of Action: Relative Contribution of Trichloroethylene (TCE) Metabolites

Several metabolites of TCE have also been shown to induce liver cancer in rodents with DCA and TCA having been the focus of study as potential active agent(s) of TCE liver toxicity and/or carcinogenesis and both able to induce peroxisome proliferation (Caldwell and Keshava, 2006). A variety of DCA effects from exposure have been noted that are consistent with conditions that increase risk of liver cancer (e.g., effects on the cytosolic enzyme glutathione [GST]-S-transferase-zeta, diabetes, and glycogen storage disease), with the pathological changes induced by DCA on whole liver consistent with changes observed in preneoplastic foci from a variety of agents (Caldwell and Keshava, 2006). Chloral hydrate (CH) is one of the first metabolites from oxidative metabolism of TCE with a large fraction of TCE metabolism appearing to go through CH and then subsequent metabolism to TCA and trichloroethanol (Chiu et al., 2006b). Similarities in toxicity may indicate that common downstream metabolites may be toxicologically important, and differences may indicate the importance of other metabolic pathways.

Although both induce liver tumors, DCA and TCA have distinctly different actions (Keshava and Caldwell, 2006) and apparently differ in tumor phenotype (see discussion above in

Section E.2.2.8) and many studies have been conducted to try to elucidate the nature of those differences (Caldwell et al., 2008b). Limitations of all of the available chronic studies of TCA and most of the studies of DCA include less than lifetime exposures, varying and small numbers of animals examined, and few exposure concentrations that were relatively high.

E.2.3.1. *Acute studies of Dichloroacetic Acid (DCA)/Trichloroacetic Acid (TCA)*

The studies in this section focus on studies of DCA and TCA that examine, to the extent possible, similar endpoints using similar experimental designs as those of TCE examined above and that give insight into proposed MOAs for all three. Of note for any experiment involving TCA, is whether exposure solutions were neutralized. Unbuffered TCA is commonly used as a reagent to precipitate proteins so that any result from studies using unbuffered TCA could potentially be confounded by the effects on pH.

E.2.3.1.1. ***Sanchez and Bull, 1990.*** In this report TCA and DCA were administered to male B6C3F1 mice (9 weeks of age) and male and female Swiss-Webster mice (9 weeks of age) for up to 14 days. At 2, 4, or 14 days, mice were injected with tritiated thymidine. Experiments were replicated at least once but results were pooled so that variation between experiments could not be determined. B6C3F1 male mice were given DCA or TCA at 0, 0.3 g/L, 1.0 g/L, or 2.0 g/L in drinking water ($n = 4$ for each group for 2 and 5 days, but $n = 15$ for control and $n = 12$ for treatment groups at Day 14). Swiss-Webster mice ($n = 4$) at were exposed to DCA only on Day 14 at 0, 1.0 or 2.0 g/L. Mice were injected with tritiated thymidine 2 hours prior to sacrifice. The pH of the drinking water was adjusted to 6.8−7.2 with sodium hydroxide. Concentrations of TCA and DCA were reported to be stable for a minimum of 3 weeks. Hepatocyte diameters were reported to be determined by randomly selecting 5 different high power fields (400×) in five different sections per animals (total of 25 fields/animal with "cells in and around areas of necrosis, close to the edges of the section, or displaying mitotic figures were not included in the cell diameter measurements." PAS staining was reported to be done for glycogen and lipofuscin determined by autofluorescence. Tritiated thymidine was reported to be given to the animals 2 hours prior to sacrifice. In 2 of 3 replications of the 14-day experiment, a portion of the liver was reported to be set aside for DNA extraction with the remaining group examined autoradiographically for tritiated thymidine incorporation into individual hepatocytes. Autoradiographs were also reported to be examined in the highest dose of either DCA or TCA for the 2- and 5-day treatment groups. Autoradiographs were reported to be analyzed in randomly selected fields (5 sections per animal in 10 different fields) for a total of 50 fields/animal and reported as percentage of cells in the fields that were labeled. There was no indication by the authors that they characterized differing zones of the liver for preferential labeling. DNA thymidine incorporation results were not examined in the same animals as those for individual hepatoctye incorporation and also not examined at 2- or 5-day time periods. The only analyses reported for the Swiss-Webster mice were of hepatic weight change and histopathology. Variations in results were reported as standard error of the mean.

Liver weights were reported but not body weights so the relationship of liver/body weight ratio could not be determined for the B6C3F1 mice. For liver weight, the numbers of animals examined varied greatly between and within treatment groups. The number of control animals examined were reported to be $n = 4$ on Day 2, $n = 8$ on day 5 and $n = 15$ on Day 14. There was also a large variation between control groups in regard to liver weight. Control liver weights for Day 2 were reported to be 1.3 ± 0.1, Day 5 to be 1.5 ± 0.05 and for Day 14 to be 1.3 ± 0.04 g. Liver weights in Day 5 control animals were much greater than those for Day 2 and Day 14 animals and thus, the means varied by as much as 15%. For DCA, there was no reported change in liver weights compared to controls values at any exposure level of DCA after 2 days of exposure. After 5 days of exposure there was no difference in liver weight between controls and 0.3 g/L exposed animals. However, the animals exposed at 1.0 or 2.0 g/L DCA had identical increases in liver weight of 1.7 ± 0.13 and 1.7 ± 0.8 g, respectively. Due to the low power of the experiment, only the 2.0 g/L DCA result was identified by the authors as significantly different from the control value. For TCA there was a slight decrease reported between control values and the 0.3 g/L treatment group (1.2 ± 0.1 g vs. 1.3 ± 0.1 g) but the 1.0 and 2.0 g/L treatment groups had similar slight increases over control (for 1.0 g/L liver weight was 1.5 ± 0.1 and for 2.0 g/L liver weight was 1.4 ± 0.1 g). The same pattern was apparent for the 5-day treatment groups for TCA as for the 2-day treatment groups.

For 14 days exposure periods the number of animals studied was increased to12 for the TCA and DCA treatment groups. After 14 days of DCA treatment, there was a reported dose-related increase in liver weight that was statistically significant at the two highest doses (i.e., at 0.3 g/L DCA liver weight was 1.4 ± 0.04, at 1.0 g/L DCA liver weight was 1.7 ± 0.07 g, and at 2.0 g/L DCA liver weight was 2.1 ± 0.08 g). This was 1.08-, 1.31-, and 1.62-fold of controls, respectively. After 14 days of TCA exposure there was a dose-related increase in liver weight that the authors reported to be statistically significant at all exposure levels (i.e., at 0.3 g/L liver weight was 1.5 ± 0.06, at 1.0 g/L liver weight was 1.6 ± 0.07 g, and at 2.0 g/L liver weight was 1.8 ± 0.10 g). This represents 1.15-, 1.23-, and 1.38-fold of control. The authors note that at 14 days that DCA-associated increases in hepatic liver weight were greater than that of TCA. What is apparent from these data are that while the magnitude of difference between the exposures was ~6.7-fold between the lowest and highest dose, the differences between TCA exposure groups for change in liver weight was ~2.5. For DCA the slope of the dose-response curve for liver weight increases appeared to be closer to the magnitude of difference in exposure concentrations between the groups (i.e., a difference of 7.7-fold between the highest and lowest dose for liver weight induction). Given that the control animal weights varied as much as 15%, the small number of animals examined, and that body weights were also not reported, there are limitations for making quantitative comparisons between TCA and DCA treatments. However, after 14 days of treatment it is apparent that there was a dose-related increase in liver weight after either DCA or TCA exposure at these exposure levels. For male and female Swiss-Webster mice 1 g/L and 2 g/L DCA treatment ($n = 4$) was reported to also induce an increase in percent liver/body weight that was similar to the magnitude of exposure difference (see below).

Grossly, livers of B6C3F1 mice treated with DCA for 1 or 2 g/L were reported to have "pale streaks running on the surface" and occasionally, discrete, white, round areas were also observed on the surface of these livers. Such areas were not observed in TCA-treated or control B6C3F1 mice. Swiss-Webster mice were reported to have "dose-related increases in hepatic weight and hepatic/body weight ratios were observed. DCA-associated increases in relative hepatic weights in both sexes were comparable to those in B6C3F1 mice. Pale streaks on the surface of the liver were not observed in Swiss-Webster mice. Again there was no significant effect on total body or renal weights (data not shown)." The authors report liver weights for the Swiss-Webster male mice ($n = 4$ for each group) to be 2.1 ± 0.1 g for controls, 2.1 ± 0.1 g for 1.0 g/L DCA and 2.4 ± 0.2 g for 2.0 g/L DCA 14-day treatment groups. The percent liver/body weights for these same groups were reported to be 6.4% ± 0.4%, 6.9% ± 0.2%, and 8.1% ± 0.3%, respectively. For female Swiss-Webster mice ($n = 4$ for each group) the liver weights were reported to be 1.1 ± 0.1 g for controls, 1.5 ± 0.1 g for 1.0 g/L DCA and 1.7 ± 0.2 g for 2.0 g/L DCA 14-day treatment groups. The percent liver/body weights for these same groups of Swiss mice were reported to be 4.8% ± 0.2%, 6.0% ± 0.2%, and 6.8% ± 0.4%, respectively. Thus, while there was no significant difference in "liver weight" between the control and the 1.0 g/L DCA treatment group for male or female Swiss-Webster mice, there was a statistically significant difference in liver/body weight ratio reported by the authors. These data, illustrate the importance of reporting both measures and the limitations of using small numbers of animals ($n = 4$ for the Swiss Webster vs. $n = 12-14$ for B6C3F1 14-days experiments). Relative liver weights were reported by the authors for male B6C3F1 mice only for the 14-day groups, as a function of calculated mean water consumption, as pooled data from the three experiments, and as a figure that was not comparable to the data reported for Swiss-Webster mice. The liver weight data indicate that male mice of the same age appeared to differ in liver weight between the two strains without treatment (i.e., male B6C3F1 mice had control liver weights at 14 days of

1.3 ± 0.04 g for 15 mice, while Swiss-Webster mice had control values of 2.1 ± 0.1 for 4 mice). While the authors report that results were "comparable" between the B6C3F1 mice in regard to DCA-induced changes in liver weight, the increase in percent liver/body weight ratios were 1.27-fold of control for Swiss-Webster male mice ($n = 4$) and 1.42-fold of control for female while the increase in liver weight for B6C3F1 male mice ($n = 12-14$) was 1.62-fold of controls after 14 days of exposure to 2 g/L DCA.

The concentration of DNA in the liver was reported as mg hepatic DNA/g of liver. This measurement can be associated with hepatocellular hypertrophy when decreased, or increased cellularity (of any cell type), increased DNA synthesis, and/or increased hepatocellular ploidy in the liver when increased. The number of animals examined for this parameter varied. For control animals there were 4 animals reported to be examined at 2 days, 8 animals examined at 5 days, and at 14 days 8 animals were examined. The mean DNA content in control livers were not reported to vary greatly, however, and the variation between animals was relatively low in the 5- and 14-day control groups (i.e., 1.67 ± 0.27 mg DNA/g, 1.70 ± 0.05 mg DNA/g, and 1.69 mg DNA/g, for 2-, 5-, or 14-day control animals, respectively). For treatment groups the number of animals reported to be examined appeared to be the same as the control animals. For DCA treatment there did not appear to be a dose-response in hepatic DNA content with the 1 g/L exposure level having the same reported value as control but the 0.3 g/L and 2.0 g/L values reported to be lower (mean values of 1.49 and 1.32 mg DNA/g, respectively). After 5 days of exposure, all treatment groups were reported to have a lower DNA content that the control value (i.e., 1.44 ± 0.06 mg DNA/g, 1.47 ± mg DNA/g, and 1.30 ± 0.14 mg DNA/g, for 0.3, 1.0, and 2.0 g/L exposure levels of DCA, respectively). After 14 days of exposure, there was a reported increase in hepatic DNA at the 0.3 g/L exposure level but significant decreases at the 1.0 g/L and 2.0 g/L exposure levels (i.e., 1.94 ± 0.20 mg DNA/g, 1.44 ± 0.14 mg DNA/g, and 1.19 ± 0.16 mg DNA/g for the 0.3, 1.0, and 2.0 g/L exposure levels of DCA, respectively). Changes in DNA concentration in the liver were not correlated with the pattern of liver weight increases after DCA treatment. For example, while there was a clear dose-related increase in liver weight after 14 days of DCA treatment, the 0.3 g/L DCA exposed group was reported to have a higher rather than lower level of hepatic DNA than controls. After 2 or 5 days of DCA treatment, liver weights were reported to be the same between the 1.0 and 2.0 g/L treatment groups but hepatic DNA was reported to be decreased.

For TCA, there appeared to be a dose-related decrease in reported hepatic DNA after 2 days of treatment (i.e., 1.63 ± 0.07 mg DNA/g, 1.53 ± 0.08 mg DNA/g, and 1. 43 ± 0.04 mg DNA/g for the 0.3 g/L, 1.0 g/L, and 2.0 g/L exposure levels of TCA, respectively). After 5 days of TCA exposure there was a reported decrease in hepatic DNA for all treatment groups that was similar at the 1.0 g/L and 2.0 g/L exposure groups (i.e., 1.45 ± 0.17 mg DNA/g, 1.29 ± 0.18 mg DNA/g, and 1. 26 ± 0.22 mg DNA/g for the 0.3 g/L, 1.0 g/L, and 2.0 g/L exposure levels of TCA, respectively). After 14 days of TCA treatment, there was a reported decrease in all treatment groups in hepatic DNA content that did not appear to be dose-related (i.e., 1.31 ± 0.17 mg DNA/g, 1.21 ± 0.17 mg DNA/g, and 1. 33 ± 0.18 mg DNA/g for the 0.3 g/L, 1.0 g/L, and 2.0 g/L exposure levels of TCA, respectively). Thus, similar to the results reported for DCA, the patterns of liver weight gain did not match those of hepatic DNA decrease for TCA treated animals. For example, although there appeared to be a dose-related increase in liver weight gain after 14 days of TCA exposure, there was a treatment but not dose-related decrease in hepatic DNA content.

In regard to the ability to detect changes, the low number of animals examined after 2 days of exposure (*n* = 4) limited the ability to detect a significant change in liver weight and hepatic DNA concentration. For hepatic DNA determinations, the larger number of animals examined at 5 and 14 day time points and the similarity of values with relatively smaller standard error of the mean reported in the control animals made quantitative differences in this parameter easier to determine. However, animals varied in their response to treatment and this variability exceeded that of the control groups. For DCA results reported at 14 days and those for TCA reported at 5 and 14 days, the standard errors for treated animals showed a much greater variability than those of the control animals (range of 0.04−0.05 mg DNA/g for control groups, but ranges of 0.17 to 0.22 mg DNA/g for TCA at 5 days and 0.14 to 0.20 mg DNA/g for DCA or TCA at 14 days). The authors stated that

> the increases in hepatic weights were generally accompanied by decreases in the concentration of DNA. However, the only clear changes were in animals treated with DCA for 5 or 14 days where the ANOVAs were clearly significant (P<0.020 and 0.005, respectively). While changes of similar magnitude were observed in other groups, the much greater variation observed in the treated groups resulted in not significant differences by ANOVA (p = 0.41, 0.66. 0.26, 0.15 for DCA – 2 days, and TCA for 2,5, and 14 days, respectively).

The size of hepatocytes is heterogeneous and correlated with its ploidy, zone, and age of the animal (see Section E.1.1 above). The authors do not indicate if there was predominance in zone or ploidy for hepatocytes included in their analysis of average hepatocyte diameter in the random selection of 25 fields per animal (*n* = 3 to 7 animals). There appeared to be a dose-related increase in cell diameter associated with DCA exposure and a treatment but not dose-related increase with TCA treatment after 14 days of treatment. For control B6C3F1 male mice (*n* = 7) the hepatocyte diameter was reported to be 20.6 ± 0.4 microns. For mice exposed to

DCA hepatocyte diameter was reported to be 22.2 ± 0.2, 25.2 ± 0.6, and 26.0 ± 1.0 microns for 0.3 g/L, 1.0 g/L, and 2.0 g/L treated mice (*n* = 4 for each group), respectively. For mice exposed to TCA hepatocyte diameter was reported to be 22.2 ± 0.2, 22.4 ± 0.6, and 23.2 ± 0.4 microns for 0.3 g/L, 1.0 g/L, and 2.0 g/L treated mice (*n* = 4 for the 0.3 g/L and 1.0 g/L groups and *n* = 3 for the 2.0 g/L group), respectively. The small number of animals examined limited the power of the experiment to determine statistically significant differences with the authors reporting that only the 1.0 g/L DCA and 2.0 g/L DCA and TCA treated groups statistically significant from control values. The dose-related increases in reported cell diameter were consistent with the dose-related increases in liver weight reported for DCA after 14 days of exposure. However, the pattern for hepatic DNA content did not. For TCA, the dose-related increases in cell diameter were also consistent with the dose-related increases in liver weight after 14 days of exposure. Similar to DCA results, the changes in hepatic DNA content did not correlate with changes in cell size. In regard to the magnitude of increases over control values, the 68 versus 38% increase in liver weight for DCA versus TCA at 2.0 g/L, was less than the 26 and 13% increases in cell diameter for the same groups, respectively. Therefore, for both DCA and TCA exposure there appeared to be dose-related hepatomegaly and increased cell size after 14-days of exposure.

The authors reported PAS staining for glycogen content as an attempt to examine the nature of increased cell size by DCA and TCA. However, they did not present any quantitative data and only provided a brief discussion. The authors reported that

> hepatic sections of DCA-treated B6C3F1 mice (1 and 2 g/L) contained very large amounts of perilobular PAS-positive material within hepatocytes. PAS stained hepatic sections from animals receiving the highest concentration of TCA displayed a much less intense staining that was confined to periportal areas. Amylase digesting confirmed the majority of the PAS-positive material to by glycogen. Thus, increased hepatocellular size in groups receiving DCA appears to be related to increased glycogen deposition. Similar increases in glycogen deposition were observed in Swiss-Webster mice.

There is no way to discern whether DCA-induced glycogen deposition was dose-related and therefore, correlated with increased liver weight and cell diameter. While the authors suggest that Swiss-Webster mice displayed “similar increased in glycogen deposition” the authors did not report a similar increase in liver weight gain after DCA exposure at 14 days (1.27-fold of control percent liver/body weight ratio in Swiss male mice and 1.42-fold in female Swiss-Webster mice vs. 1.62-fold of control in B6C3F1 mice after 14 days of exposure to 2 g/L DCA). Thus, the contribution of glycogen deposition to DCA-induced hepatomegaly and the nature of increased cell size induced by acute TCA exposure cannot be determined by this study.

However, this study does show that DCA and TCA differ in respect to their effects on glycogen deposition after short-term exposure and the data suggest that.

The authors report that

> localized areas of coagulative necrosis were observed histologically in both B6C3F1 and Swiss-Webster mice treated with DCA at concentrations of 1 and 2 g/L for 14 days. The necrotic areas corresponded to the pale streaked areas seen grossly. These areas varied in size, shape and location within sections and occupied up to several mm^2. An acute inflammatory response characterized by thin rims of neutrophils was associated with the necrosis, along with multiple mitotic figures. No such areas of necrosis were observed in animals treated at lower concentrations of DCA, or in animals receiving the chemical for 2 or 5 days. Mice treated with 2 g/L TCA for 14 days have some necrotic areas, but at such low frequency that it was not possible to determine if it was treatment-related (2 lesions in a total of 20 sections examined). No necrosis was observed in animals treated at the lower concentrations of TCA or at earlier time points.

Again there were no quantitative estimates given of the size of necrotic areas, variation between animals, variation between strain, or dose-response of necrosis reported for DCA exposure by the authors. The lack of necrosis after 2 and 5 days of exposure at all treatment levels and at the lower exposure level at 14 days of exposure is not correlated with the increases in liver weight reported for these treatment groups.

Autoradiographs of randomly chosen high powered fields (400×) (50 fields/animal) were reported as the percentage of cells in the fields that were labeled. There was significant variation in the number of animals examined and in the reported mean percent of labeled cells between control groups. The number of control animals was not given for the 2-day group but for the 5-day and 14 day groups were reported to be $n = 4$ and $n = 11$, respectively. The mean percent of labeling in control animals was reported at 0.11 ± 0.03, 0.12 ± 0.04, and 0.46 ± 0.07% of hepatocytes for 2-day, 5-day, and 14-day control groups, respectively. Only the 2.0 g/L exposures of DCA and TCA were examined at all 3 times of exposure while all groups were examined at 14 days. However, the number of animals examined in all treatment groups appeared to be only 4 animals in each group. There was not an increase over controls reported in the 2.0 g/L DCA or TCA 2- and 5-day exposure groups in hepatocyte labeling with tritiated thymidine. After 14 days of exposure, there was a statistically significant but very small dose-related increase over the control value after DCA exposure (i.e., 0.46% ± 0.07%, 0.64% ± 0.15%, 0.75% ± 0.22%, and 0.94% ± 0.05% labeling of hepatocytes in control, 0.3, 1.0, and 2.0 g/L DCA treatment groups, respectively). For TCA, there was no change in hepatocyte labeling except for a 50% decrease from control values at after 14 days of exposure to 2.0 g/L

TCA (i.e., 0.46% ± 0.07%, 0.50% ± 0.14%, 0.52% ± 0.26%, and 0.26% ± 0.14% labeling of hepatocytes in control, 0.3, 1.0, and 2.0 g/L TCA treatment groups, respectively). The authors report that

> labeled cells were localized around necrotic areas in these [sic DCA treated] groups. Since counts were made randomly, the local increased in DCA-treated animals at concentrations of 1 and 2 g/L are in fact much higher than indicated by the data. Labeling indices in these areas of proliferation were as high as 30%. Labeled hepatocytes in TCA-treated and the control animals were distributed uniformly throughout the sections. There was an apparent decrease in the percentage of labeled cells in the group of animals treated with the highest dose of TCA. This is because no labeled cells were found in any of the fields examined for one animal.

The data for control mice in this experiment is consistent with others showing that the liver is quiescent in regard to hepatocellular proliferation with few cells undergoing mitosis (see Section E.1.1). For up to 14 days of exposure with either DCA or TCA, there is little increase in hepatocellular proliferation except in instances and in close proximity to areas of proliferation. The increases in liver weight reported for this study were not correlated with and cannot be a result of hepatocellular proliferation as only a very small population of hepatocytes is undergoing DNA synthesis. For TCA, there was no increase in DNA synthesis in hepatocytes, even at the highest dose, as shown by autoradiographic data of tritiated thymidine incorporation in random fields.

Whole liver sections were examined for tritiated thymidine incorporation from DNA extracts. The number of animals examined varied (i.e., $n = 4$ for the 2-day exposure groups and $n = 8$ for 5- and 14-day exposure groups) but the number of control animals examined were the same as the treated groups for this analysis. The levels of tritiated thymidine incorporation in hepatic DNA (dpm/mg DNA expressed as mean x 10^3 ± SE of n animals) were reported to be similar across control groups (i.e., 56 ± 11, 56 ± 6, and 56 ± 7 dpm/mg DNA, for 2-, 5-, and 14-day treatment groups, respectively). After two days of DCA exposure, there appeared to be a slight treatment-related but not dose-related increase in reported tritiated thymidine incorporation into hepatic DNA (i.e., 72 ± 23, 80 ± 6, and 68 ± 7 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L DCA, respectively). After 5 days of DCA exposure, there appeared to be a dose-related increase in reported tritiated thymidine incorporation into hepatic DNA (i.e., 68 ± 18, 110 ± 20, and 130 ± 7 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L DCA, respectively). However, after 14 days of DCA exposure, levels of tritiated thymidine incorporation were less than those reported at 5 days and the level for the 0.3 g/L exposure group was less than the control value (i.e., 33 ± 11, 77 ± 9, and 81 ± 12 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L DCA, respectively). After two days of TCA exposure there did not appear to be a treatment-related increase in tritiated thymidine incorporation into hepatic DNA (i.e., 82 ± 16, 52 ± 7, and 54 ± 7 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L TCA, respectively). Similar to the reported results for DCA, after 5 days of TCA exposure there appeared to be a dose-related increase in reported tritiated thymidine incorporation into hepatic DNA (i.e., 79 ± 23, 86 ± 17, and 158 ± 33 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L TCA, respectively). After 14 days of TCA exposure there were treatment related increases but not a dose-related increase in reported tritiated thymidine incorporation into hepatic DNA (i.e., 71 ± 10, 73 ± 14, and 103 ± 14 dpm/mg DNA for 0.3, 1.0, or 2.0 g/L TCA, respectively). It would appear that for both TCA and DCA the increase in tritiated thymidine incorporation into hepatic DNA was dose related and peaked after 5 days of exposure. The authors report that the decrease in incorporation into hepatic DNA observed after 14 days of DCA treatment at 0.3 g/L to be statistically significant as well as the increases after 5 and 14 days of TCA exposure at the 2.0 g/L level. The small numbers of animals examined, the varying number of animals examined, and the degree of variation in treatment-related effects limits the statistical power of this experiment to detect quantitative changes.

Given the limitations of this experiment, determination of an accurate measure of the quantitative differences in tritiated thymidine incorporation into whole liver DNA or that observed in hepatocytes are hard to determine. In general the results for tritiated thymidine incorporation into hepatic DNA were consistent with those for tritiated thymidine incorporation into hepatocytes in that they show that there were at most a small population of hepatocytes undergoing DNA synthesis after up to 14 days of exposure at relative high levels of exposure to DCA and TCA (i.e., the largest percentage of hepatocytes undergoing DNA synthesis for any treatment group was less than 1% of hepatocytes). The highest increases over control levels for hepatic DNA incorporation for the whole liver were reported at the highest exposure level of TCA treatment after 5 days of treatment (3-fold of control) and after 14 days of TCA treatment (2-fold of control). Although the authors report small areas of focal necrosis with concurrent localized increases in hepatocyte proliferation in DCA treated animals exposed to1.0 g/L and 2.0 g/L DCA, the levels of whole liver tritiated thymidine incorporation were only slightly elevated over controls at these concentrations, and were decreased at the 0.3 g/L exposure concentration for which no focal necrosis was reported. The whole liver DNA incorporation of tritiated thymidine was not consistent with the pattern of tritiated thymidine incorporation observed in individual hepatocytes. The authors state that "at present, the mechanisms for increased tritiated thymidine uptake in the absence of increased rates of cell replication with increasing doses of TCA cannot be determined." The authors do not discuss the possibility that the difference in hepatocyte labeling and whole liver DNA tritiated thymidine incorporation could have been due to the labeling representing increased polyploidization rather than cell proliferation, as well as increased numbers of proliferating nonparenchymal and inflammatory cells. The increased cell size due from TCA exposure without concurrent increased glycogen deposition could have been indicative of increased polyploidization. Finally, although both TCA- and DCA-induced increases in liver weight were generally consistent with cell size increases, they were not correlated with patterns of change in hepatic DNA content, incorporation of tritiated thymidine in DNA extracts from whole liver, or incorporation of tritiated thymidine in hepatocytes. In regard to cell size, although increased glycogen deposition with DCA exposure was noted by the authors of this study, lack of quantitative analyses of that accumulation precludes comparison with DCA-induced liver weight gain.

E.2.3.1.2. ***Nelson et al., 1989.*** Nelson and Bull (1988) administered TCE (0, 3.9, 11.4, 22.9, and 30.4 mmol/kg) in Tween 80® via gavage to male Sprague Dawley rats and male B6C3F1 mice, sacrificed them fours hours after treatment (n = 4−7), and measured the rate of DNA unwinding under alkaline conditions. They assumed that this assay represented increases in single-strand breaks. For rats there was little change from controls up to 11.4 mmol/kg (1.5 g/kg TCE) but a significantly increased rate of unwinding at 22.9 and 30.4 mmol/kg TCE (~2-fold greater at 30.4 mmol). For mice there was a significantly increased level of DNA unwinding at 11.4 and 22.9 mmol. Concentrations above 22.9 mmol/kg were reported to be lethal to the mice. In this same study, TCE metabolites were administered in unbuffered solution using the same assay. DCA was reported to be most potent in this assay with TCA being the lowest, while CH closely approximated the dose-response curve of TCE in the rat. In the mouse the most potent metabolite in the assay was reported to be TCA followed by DCA with CH considerably less potent.

The focus of the Nelson et al. (1989) study was to examine whether reported single strand breaks in hepatic DNA induced by DCA and TCA (Nelson and Bull, 1988) were secondary to peroxisome proliferation also reported to be induced by both. Male B6C3F1 mice (25−30 g but no age reported) were given DCA (10 mg/kg or 500 mg/kg) or TCA (500 mg/kg) via gavage in 1% aqueous Tween 80® with no pH adjustment. The animals were reported to be sacrificed 1, 2, 4, or 8 hours after administration and livers examined for single strand breaks as a whole liver homogenate. In a separate experiment (experiment #2) treatment was parallel to the first (500 mg/kg treatment of DCA or TCA) but levels of PCO activity were measured as an indication of peroxisome proliferation and expressed as μmol/min/g liver. In a separate experiment (experiment #3) mice were administered 500 mg/kg DCA or TCA for 10 days with

Clofibrate administered at a dose of 250 mg/kg as a positive control. 24 hours after the last dose, animals were killed and liver examined by light microscopy and PCO activity. Finally, in an experiment parallel in design to experiment #3, single strand breaks were measured in total hepatic DNA after 500 mg/kg exposure to TCA (experiment #4). Electron microscopy was performed on 2 animals/group for vehicle, DCA or TCA treatment, with 6 randomly chosen micrographic fields utilized for peroxisome profiles. These micrographs were analyzed without identification as to what area of the liver lobules they were being taken from. Hence there is a question as to whether the areas which are known to be peroxisome rich were assayed of not.

The data from all control groups were reported as pooled data in figures but statistical comparisons were made between concurrent control and treated groups. The results for DNA single strand breaks were reported for "13 control animals" and each experimental time point "as at least 6 animals." DNA strand breaks were reported to be significantly increased over concurrent control by a single exposure to 10 or 500 mg/kg DCA or 500 mg/kg TCA for 1, 2, or 4 hours after administration but not at 8 or 24 hours. There did not appear to be a difference in the magnitude of response between the 3 treatments (the fraction of unwound DNA was ~2.5 times that of control). PCO activity was reported to be not increased over control within 24 hours of either DCA or TCA treatment. (n = 6 animals per group). The fraction of alkaline unwinding rates as an indicator of single strand breaks were reported to not be significantly different from controls and TCA-treated animals after 10 days of exposure (n = 5).

Relative to controls, body weights were reported to not be affected by exposures to DCA or TCA for 10 days at 500 mg/kg (data were not shown.) (n = 6 per group). However, both DCA and TCA were reported to significantly increase liver weight and liver/body weight ratios (i.e., liver weights were 1.3 ± 0.05 g, 2.1 ± 0.10 g, and 1.7 ± 0.09 g for control, 500 mg/kg DCA and 500 mg/kg TCA treatment groups, respectively while percent liver/body weights were 4.9% ± 0.14%, 7.5% ± 0.18%, and 5.7% ± 0.14% for control, 500 mg/kg DCA and 500 mg/kg TCA treatment groups, respectively). PCO activity (μmol/min/g liver) was reported to be significantly increased by DCA (500 mg/kg), TCA (500 mg/kg), and Clofibrate (250 mg/kg) treatment (i.e., levels of oxidation were 0.63 ± 0.07, 1.03 ± 0.09, 1.70 ± 0.08, and 3.26 ± 0.05 for control, 500 mg/kg DCA, 500 mg/kg TCA and 250 mg/kg Clofibrate treatment groups, respectively). Thus, the increases were ~1.63-, 2.7-, and 5-fold of control for DCA, TCA and Clofibrate treatments. Results from randomly selected electron photomicrographs from 2 animals (6 per animal) were reported for DCA and TCA treatment and to show an increase in peroxisomes per unit area that was reported to be statistically significant (i.e., 9.8 ± 1.2, 25.4 ± 2.9, and 23.6 ± 1.8 for control, 500 mg/kg DCA and 500 mg/kg TCA, respectively). The 2.5- and 2.4-fold of control values for DCA and TCA gave a different pattern than that of PCO activity. The small number of animals examined limited the power of the experiment to quantitatively determine the magnitude of peroxisome proliferation via electron microscopy. The enzyme analyses suggested that both DCA and TCA were weaker inducers of peroxisome proliferation that Clofibrate.

The authors report that there was no evidence of gross hepatotoxicity in vehicle or TCA-treated mice. Light microscopic sections from mice exposed to TCA or DCA for 10 days were stained with H&E and PAS for glycogen. For TCA treatment, PAS staining "produced approximately the same intensity of staining and amylase digesting revealed that the vast majority of PAS-positive staining was glycogen." Hepatocytes were reported to be "slightly larger in TCA-treated mice than hepatocytes from control animals throughout the liver section with the architecture and tissue pattern of the liver intact." The histopathology after DCA treatment was reported to be "markedly different than that observed with either vehicle or TCA treatments" with the "most pronounced change in the size of hepatocytes." DCA was reported to

> produce marked cellular hypertrophy uniformly throughout the liver. The hepatocytes were approximately 1.4 times larger in diameter than control liver cells. This hypertrophy was accompanied by an increase in PAS staining; indicating greater glycogen deposition than in TCA-treated and control liver tissue. Multiple white streaks were grossly visible on the surface of the liver of DCA-treated mice. The white areas corresponded with subcapsular foci of coagulative necrosis. These localized necrotic areas were not encapsulated and varied in size. The largest necrotic foci occupied the area of a single lobule. These necrotic areas showed a change in staining characteristics. Often this change consisted of increased eosinophilia. A slight inflammatory response, characterized by neutrophil infiltration, was present. These changed were evident in all DCA-treated mice.

The results from this experiment cannot inform as to dose-response relationships for the parameters tested with the exception of DNA single strand breaks where 2 concentrations of DCA were examined (10 and 500 mg/kg). For this parameter the 10 mg/kg exposure of DCA was as effective as the 500 mg/kg dose where toxicity was observed. This effect on DNA was also observed before evidence of induction of peroxisome proliferation. The authors did not examine Clofibrate for effects on DNA so whether it too, would have produced this effect is unclear. The results from this study are consistent with those of Sanchez and Bull (1990) for induction of hepatomegaly by DCA and TCA, the lack of hepatotoxicity at this dose by TCA, and the difference in glycogen deposition between DCA and TCA.

E.2.3.1.3. ***Styles et al., 1991.*** In this report a similar paradigm is used as Nelson et al. (1989) for the determination of repeating that work on single strand breakage and to study DNA synthesis and peroxisome proliferation. In regard to the findings of single strand breaks, Styles et al. (1991) reported for a similar paradigm of 500 mg/kg neutralized TCA administered to male B6C3F1 mice (7−8 weeks of age) examined at 1, 4, 8, and 24 hours after dosing, reported no increased unwinding of DNA 1 or 24 hours after TCA administration. In a separate experiment tritiated thymidine was administered to mice 1 hour before sacrifice at 24, 36, 48, 72, and 96 hours after the first dose of 500 mg/kg TCA for 3 days via gavage (n = 5 animals per group).

The hepatic DNA uptake of tritiated thymidine was reported to be similar to control levels up to 36 hours after the first dose and then to increase to a level ~6-fold greater than controls by 72 hours after the first dose of TCA. By 96 hours the level of tritiated thymidine incorporation had fallen to ~4-fold greater than controls. The variation, reported by standard deviation (SD) was very large in treated animals (e.g., SD was equal to approximately ±1.3-fold of control for 48 hour time point). Individual hepatocytes were examined with the number of labeled hepatocytes/1,000 cells reported for each animal. The control level was reported to be ~1 with a SD of similar magnitude. The number of labeled hepatocytes was reported to decrease between 24 and 36 hours and then to rise slowly back to control levels at 48 hour and then to be significantly increased 72 hours after the first dose of TCA (~9 cells/1,000 with a SD of 3.5) and then to decrease to a level of ~5 cells/1,000. Thus, it appears that increases in hepatic DNA tritiated thymidine uptake preceded those of increased labeled hepatocytes and did not capture the decrease in hepatocyte labeling at 36 hours. By either measure the population of cells undergoing DNA synthesis was small with the peak level being less than 1% of the hepatocyte population. The authors go on to report the zonal distribution of mean number of hepatocytes incorporating tritiated thymidine but no variations between animals were reported. The decrease in hepatocyte labeling at 36 hours was apparent at all zones. By 48 hours there appeared to be slightly more perioportal than midzonal cells undergoing DNA synthesis with centrilobular cells still below control levels. By 72 hours all zones of the liver were reported to have a similar number of labeled cells. By 96 hours the midzonal and centrilobular regions have returned almost to control levels while the periportal areas were still elevated. These results are consistent with all hepatocytes showing a decrease in DNA synthesis by 36 hours and then a wave of DNA synthesis occurring starting at the periportal zone and progressing through to the pericentral zone until 72 hours and then the midzonal and pericentral hepatocytes completing their DNA synthesis activity. Peroxisome proliferation was assessed via electron photomicrographs taken in mice (4 controls and 4 treated animals) given 10 daily doses of 500 mg/kg TCA and killed 14 hours after the last dose. No details were given by the authors as to methodology for peroxisome volume estimate (e.g., how many photos per animals were examined and whether they were randomly chosen). The mean percent cell volume occupied by peroxisome was reported to be 2.1% ± 0.386% and 3.9% ± 0.551% for control and 500 mg/kg TCA, respectively. Given there were no time points examined before 10 days for peroxisome proliferation, correlations with DNA synthesis activity induced by TCA cannot be made from this experiment. However, it is clear from this study that a wave of DNA synthesis occurs throughout the liver after treatment of TCA at this exposure concentration and that it has peaked by 72 hours even with continuous exposure to 96 hours. Whether the DNA synthesis represents polyploidization or cell proliferation cannot be determined from these data as neither can a dose-response.

E.2.3.1.4. ***Carter et al., 1995.*** The aim of this study was to "use correlative biochemical, pathologic and morphometric techniques to characterize and quantify the acute, short-term responses of hepatocytes in the male B6C3F1 mouse to drinking water containing DCA." This report used tritiated thymidine incorporation, DNA concentration, hepatocyte number per field (cellularity), nuclear size and binuclearity (polyploidy) parameters to study 0, 0.5, and 5 g/L neutralized DCA exposures up to 30 days. Male B6C3F1 mice were started on treatment at 28 days of age. Tritiated thymidine was administered by miniosmotic pump 5 days prior to sacrifice. The experiment was conduced in two phases which consisted of 5−15 days of treatment (Phase I) and 20−30 days of treatment (Phase II) with 5 animals per group in groups sacrificed at 5-day intervals. Liver sections were stained for H&E, PAS (for glycogen) or methyl green pryonin stain (for RNA). DNA was extracted from liver homogenates and the amount of tritiated thymidine determined as dpm/μg DNA. Autoradiography was performed with the number of hepatocyte nuclei scored in 1,000 hepatocytes selected randomly to provide a labeling index of "number of labeled cells/1000 X 100%." Changes in cellularity, nuclear size and number of multinucleate cells were quantified in H&E sections at 40× power. Hepatocyte cellularity was determined by counting the number of nuclei in 50 microscopic fields with multinucleate cells being counted as one cell and nonparenchymal cells not counted. Nuclear size was also measured in 200 nuclei with the mean area plus 2 SD was considered to be the largest possible single nucleus. Therefore, polyploid diploid cells were identified by the authors but not cells that had undergone polyploidy with increased DNA content in a single nucleus.

Mean body weights at the beginning of the experiment varied between 18.7 and 19.6 g in the first 3 exposure groups of Phase I of the study. Through 15 days of exposure there did not appear to be a change in body weight in the 0.5 g/L exposure groups but in the 5 g/L exposure group body weight was reduced at 5, 10 and 15 days with that reduction statistically significant at 5 and. 15 days. Liver weights did not appear to be increased at Day 5 but were increased at days 10 and 15 in both treatment groups (i.e., means ± S.E.M. for Day 10; 1.36 ± 0.03, 1.46 ± 0.03, and 1.59 ± 0.08 g for control, 0.5 and 5 g/L DCA, respectively and for Day 15; 1.51 ± 0.06, 1.72 ± 0.05, and 2.08 ± 0.11 g for control, 0.5 and 5 g/L DCA, respectively). The percent liver/body weight followed a similar pattern with the exception that at Day 5 the 5 g/L exposure group had a statistically significant increase over control (i.e., for Day 10; 6.00% ± 0.10%, 6.72% ± 0.17%, and 8.21% ± 0.10% for control, 0.5 and 5 g/L DCA, respectively and for Day 15; 6.22 ± 0.08, 6.99 ± 0.15, and 10.37 ± 0.27% g for control, 0.5 and 5 g/L DCA, respectively).

In Phase II of the study, control body weights were smaller than Phase I and varied between 16.6 and 16.9 g in the first 3 exposure groups. Liver weights of controls were also smaller making it difficult to quantitatively compare the two groups in terms of absolute liver weights. However, the pattern of DCA-induced increases in liver weight and percent liver/body weight remained. The patterns of body weight reduction only in the 5 g/L treatment groups and increased liver weight with DCA treatment at both concentrations continued from 20 to 30 days of exposure. For liver weight there was a slight but statistically significant increase in liver weight for the 0.5 g/L treatment groups over controls (i.e., for Day 20; 1.02 ± 0.02, 1.18 ± 0.05, and 1.98 ± 0.05 g for control, 0.5 and 5 g/L DCA, respectively, for Day 25; 1.15 ± 0.03, 1.34 ± 0.04, and 2.06 ± 0.12 g for control, 0.5 and 5 g/L DCA, respectively, for Day 30; 1.15 ± 0.03, 1.39 ± 0.08, and 1.90 ± 0.12 g for control, 0.5 and 5 g/L DCA, respectively). For percent liver/body weight there was a small increase at 0.5 g/L that was not statistically significant but all other treatments induced increases in percent liver/body weight that were statistically significant (i.e., for Day 20; 4.82% ± 0.07%, 5.05% ± 0.09%, and 9.71% ± 0.11% for control, 0.5 and 5 g/L DCA, respectively, for Day 25; 5.08% ± 0.04%, 5.91% ± 0.09%, and 10.38% ± 0.58% for control, 0.5 and 5 g/L DCA, respectively, for Day 30; 5.17% ± 0.09%, 6.01% ± 0.08%, and 10.28% ± 0.28% for control, 0.5 and 5 g/L DCA, respectively). Of note is the dramatic decrease in water consumption in the 5 g/L treatment groups that were consistently reduced by 64% in Phase I and 46% in Phase II. The 0.5 g/L treatment groups had no difference from controls in water consumption at any time in the study. The effects of such water consumption decreases would affect body weight as well as dose received. Given the differences in the size of the animals at the beginning of the study and the concurrent differences in liver weights and percent liver/body weight in control animals between the two phases, the changes in these parameters through time from DCA treatments cannot be accurately determined (e.g., control liver/body weights averaged 6.32% in Phase I but 5.02% in Phase II). However, percent liver/body weight increase were reported to be consistently increased within and between both phases of the study for the 0.5 g/L DCA treatment from 5 days of treatment to 30 days of treatment (i.e., for Phase I the average increase was 9.5% and for Phase II the average increased was 12.5% for 0.5 g/L DCA treated groups). Although increase at 5 days the nonsignificance of the change may be resultant from the small number of animals examined. The difference in magnitude of dose and percent liver/body weight increase is difficult to determine given that the 5 g/L dose of DCA reduced body weight and significantly reduced water consumption by ~50% in both phases of the study. Of note is that the differences in DCA-induced percent liver/body weight were ~6-fold for the 15, 25, and 30-day data between the 0.5 and 5 g/L DCA exposures rather than the 10-fold difference in exposure concentration in the drinking water.

The incorporation of tritiated thymidine into total hepatic DNA control treatment groups was reported to be 73.34 ± 11.74 dpm/μg DNA at 5 days, 34 ± 4.12 dpm/μg DNA at 15 days, and 28.48 ± 3.24 dpm/μg DNA at 20 days but was not reported for other treatments. The results for 0.5 g/L treatments were not reported quantitatively but the authors stated that the results "showed similar trends of initial inhibition followed by enhancement of labeling, the changes relative to controls were not statistically significant." For 5 g/L treatment groups the 5-day treated groups DNA tritiated thymidine incorporation was reported to be 42.8% of controls and followed by a transient increase at 15 and 20 days (i.e., 2.65- and 2.45-fold of controls, respectively) but after 25 and 30 days to not be significantly different from controls (data not shown). Labeling indices of hepatocytes were reported as means but variations as either SEM or SD were not reported. Control means were reported as 5.5, 4, 2, 2, 3.2, and 3.5% of randomly selected hepatocytes for 5, 10, 15, 20, 25, and 30 days, respectively, for 4 to 5 animals per group. In contrast to the DNA incorporation results, no increase in labeling of hepatocytes was reported to be observed in comparison to controls for any DCA treatment group from 5 to 30 days of DCA exposure. The 5 g/L treatment group showed an immediate decrease in hepatocyte labeling from Day 5 onwards that gradually increased approximately half of control levels by Day 30 of exposure (i.e., <0.5% labeling index [LI] at Day 5, ~1% LI at Day 10, ~0.6% LI at Day 20, 1% LI at Day 25 and 2% LI at Day 30). For the 0.5 g/L treatment the labeling index was reported to not differ from controls from days 5 though 15 but to be significantly decreased between days 20 and 30 to levels similar to those observed for the 5 g/L exposures. The relatively higher number of hepatocytes incorporating label reported in this study than others can be reflection of the longer times of exposure to tritiated thymidine. Here, incorporation was shown for 1 weeks worth of exposure and reflects the percent of cell undergoing synthesis during that time period. Also the higher labeling index in control animals at the 5 and 10 day exposure periods is probably a reflection of the age of the animals at the time of study. From the data reported by the authors, there was a correlation between the patterns of total DNA incorporation of label and hepatocyte labeling indices in control groups (i.e., higher level of labeling at 5 days than at 15 and 20). However, the patterns of decreased thymidine labeling reported for hepatocytes were not correlated with a transient increase in total DNA thymidine incorporation reported with DCA treatment, especially at the 5 g/L exposure level with a large decrease reported for the number of labeled hepatocytes at the same time an increase in total DNA thymidine incorporation was reported. Although reported to be transiently increased, the total hepatic DNA labeling still represented at most a 2.5-fold increase over control liver, which represents a small population of cells. Given that the study examined hepatocyte labeling in random fields and did not report quantitative zonal differences in proliferation, a more accurate determination of what hepatocytes were undergoing proliferation cannot be made from the LI results. Also although the authors report signs of inflammatory cells for 5-day treatment there is no reference to any inflammatory changes that may have been observed at later time periods when cellular degeneration and loss of nuclei were apparent. Such an increase inflammatory infiltrates can increase the DNA synthesis measurements in the liver. The difference in LI and total DNA synthesis could reflect differences in nonparenchymal cell proliferation or ploidy changes versus mitoses in hepatocytes. Clearly, the increases in liver weight that were reported as early as 5 days of exposure could not have resulted from increased hepatocyte proliferation.

The H&E sections were reported to have been fixed in an aqueous solution that reduced glycogen content. However, residual PAS positive material (assumed to be glycogen) was reported to be present indicating that not all of the glycogen had been dissolved. The authors report changes in pathology between 5 and 30 days in control animals that included straightening of hepatocyte cording, decreased mitoses, less clarity and more fine granularity of pericentral hepatocellular cytoplasm, increased numbers of larger nuclei that were not labeled, and reported differences between animals in the amount of glycogen present (i.e., 2 or 3 animals out of the 5 had less glycogen than other members of the group with less glycogen in the central and midzonal areas). These changes are consistent with increased polyploidization expected for maturing mice (see Sections E.1.1 and E.1.2 above). After 5 days of treatment, 0.5 g/L exposed animals were reported to have livers with fewer mitoses and tritiated thymidine hepatocyte labeling but by 10 days an increase in nuclear size. Labeling was reported to be predominantly in small nuclei. Animals given 0.5 g/L DCA for 15, 20, and 25 days were reported to have "focal cells in the middle zone with less detectable or no cell membranes and loss of the coarse granularity of the cytoplasm" with some cells not having nuclei or cells having a loss of nuclear membrane and apparent karyolysis. "Cells without nuclei because the plane of the section did not pass through the nuclei had the same type of nuclei. Cells without nuclei not related to plane of section had a condensed cytoplasm." Livers from 20-day and later sacrifice groups treated with 0.5 g/L DCA were reported to have normal architecture. After 25 days of treatment apoptotic bodies were reported to be observed with fewer nuclei around the central veins nuclei that were larger in central and midzonal areas. In animals treated with 5 g/L DCA the authors report similar features as for 0.5 g/L but in a zonal pattern. Inflammatory cells were reported to not be observed and after 5 and 10 days a marked decrease in labeled nuclei. After 5 days of 5 g/L DCA, nuclear depletion in the central and mid-zonal areas was reported. In methyl green pyronin-stained slides a marked loss of cellular membranes was reported at 5 days with a loss of nuclei and formation of "lakes of liver cell debris." After 15 days of treatment there was a reported increase in labeling in comparison to animals sacrificed after 5 or 10 days. The cells nearest to the triads were reported to have clearing of their cytoplasms and an increase in PAS positivity. Hepatocytes of both 0.5 and 5 g/L DCA treatment groups were reported to have "enlarged, presumably polyploidy nuclei." Some of the nuclei were reported to be "labeled, usually in hepatocytes in the mid-zonal area."

The morphometric analyses of liver sections were reported to reveal statistically significant changes in cellularity, nuclear size (as measured by either nuclear area or mean diameter of the nuclear area equivalent circle), and multinucleated cells during 30 days exposure to DCA. The authors reported that the concentration of total DNA in the liver, reported as total μg nuclear DNA/g liver, ranged between 278.17 ± 16.88 and 707.00 ± 25.03 in the control groups (i.e., 2−5-fold range). No 0.5 g/L DCA treatment groups differed from their control group in terms of liver DNA concentration. However, for 10 though 30 days of exposure hepatic DNA concentrations were reported to be decreased in the 5 g/L treatment groups (at 5 days there appeared to be ~30% increase over control). The number of cells per field was reported to range between 24.28 ± 1.94 and 43.81 ± 1.93 in control livers (i.e., 1.8-fold range). From 5 to 15 days the number of cells/field decreased with 0.5 g/L DCA treatment although only at Day 15 was the change statistically significant. From 20 to 30 days of treatment only the 30 day treatment showed a slight decrease in cells/field and that change was statistically significant. After 5 days of treatment, the number of cells/field was 1.6-fold of control, by 15 days reduced by ~20%, and for 20 to 30 days continued to be reduced by as much as 40%. Although the authors reported that the changes in cellularity and DNA concentration to be closely correlated, the patterns in the number of cells/field varied in their consistency with those of DNA concentration (i.e., for days 5, 20 and 25 there direction of change with dose was similar between the two parameters but for days 10, 15 and 30 were not). If changes in liver weight were due to hepatocellular hypertrophy, the increased liver size would be matched by a decrease in liver DNA concentration and by the number of cells/field. The large increases in liver/body weight induced by 5 g/L DCA were matched by decreases in liver DNA concentration except for the 5 day exposure group. In general, the small increases in liver/body weight consistently induced by 0.5 g/L treatment from

Day 5 through 30 were not correlated with DNA concentrations or cells/field. The small number of animal examined for these parameters (i.e., $n = 4-5$) and the highly variable control values limit the power to accurately detect changes. The apparent dehydration in the animals treated at 5 g/L DCA was cited by the authors for the transient increase in cellularity and DNA concentration in the 5-day exposure group. However, drinking water consumption was reported to be similarly reduced at all treatment periods for 5 g/L DCA-treated animals so that all groups would experience the same degree of dehydration.

The percentage of mononucleated cells was reported as percent of mononucleated hepatocytes with results given as means but with no reports of variation within groups. The mean control values were reported to range between 60 and 75% for Phase I and between 58 and 71% for Phase II of the experiment ($n = 4-5$ animals per group). The percent of mononucleated hepatocytes was reported to be similar between control and DCA treatment groups at 5- and 10-day exposure. At 15 days both DCA treatments were reported to give a similar increase in mononucleated hepatocytes (~80 vs. 60% in control) with only the 5 g/L DCA group statistically significant. The increase in mononucleated cells reported for DCA treatment is similar in size to the variation between control values. For Phase II of the study, DCA treatment was reported to increase the number of mononucleated cells in at all concentrations and exposure time periods in comparison to control values. However, only the increases for the 5 g/L treatments at days 20 and 25, and the 0.5 g/L treatment at Day 30 were reported to be statistically significant. Again, small numbers of animals limit the ability to accurately determine a change. However, the consistent reporting of an increasing number of mononucleated cells between 15 and 30 days could be associated with clearance of mature hepatocytes as suggested by the report of DCA-induced loss of cell nuclei.

Mean nuclear area was reported to range between 45 and 54 μ^2 in Phase I and to range between 41 and 48 μ^2 in Phase II of the experiment with no variation in measurements given by the authors. The only statistically significant differences reported between control and treated groups in Phase I was a decrease from 54 to ~42 μ^2 in the 0.5 g/L DCA 10 day treatment group and a small increase from 50 to ~52 μ^2 15 day treatment group. Clearly the changes reported by the authors as statistically significant did not show a dose-related pattern and were within the range of variation reported between control groups. For Phase II of the experiment both DCA treatment concentrations were reported to induce a statistically significant increase the nuclear area that was dose-related with the exception of Day 30 in which the nuclear area was similar between the 0.5 and 5 g/L treatment groups. The largest increase in nuclear area was reported at 20 days for the 5 g/L treatment group (~72 vs. 41 μ^2 for control). The patterns of increases in nuclear area were correlated with those of increased percentage of mononucleated cells in

Phase II of the study (20−30 days of treatment) as well as the small changes seen in Phase I of the experiment. An increase in nuclear cell area is consistent with increase polyploidization without mitosis as cells are induced towards polyploidization. A decrease in the numbers of binucleate cells in favor of mononucleate cells is consistent with clearance of mature binucleate hepatocyte as well induction of further polyploidization of diploid or tetraploid binucleate cell to tetraploid or octoploid mononucleate cells. The authors suggested that the "large hyperchromatic mononucleated hepatocytes are tetraploid" and suggest that such increases in tetraploid cells have also been observed with nongenotoxic carcinogens and with di(2-ethylhexyl) phthalate (DEHP). In terms of increased cellular granularity observed by the authors with DCA treatment, this result is also consistent with a more differentiated phenotype (Sigal et al., 1999). Thus, these results for DCA are consistent with a DCA induced change in polyploidization of the cells without cell proliferation. The pattern of consistent increase in percent liver/body weight induced by 0.5 g/L DCA treatment from days 5 though 30 was not consistent with the increased numbers of mononucleate cells and increase nuclear area reported from Day 20 onward. The large differences in liver weight induction between the 0.5 g/L treatment group and the 5 g/L treatment groups at all times studied also did not correlate with changes in nuclear size and percent of mononucleate cells. Thus, increased liver weight was not a function of cellular proliferation, but probably included both aspects of hypertrophy associated with polyploidization and increased glycogen deposition induced by DCA. The similar changes reported after short-term exposure for both the 0.5 and 5 g/L exposure concentration were suggested by the authors to indicate that the carcinogenic mechanism at both concentrations would be similar. Furthermore, they suggest that although there is evidence of cytotoxicity (e.g., loss of cell membranes and apparent apoptosis), the present study does not support that the mechanism of DCA-induced hepatocellular carcinogenesis is one of regenerative hyperplasia following massive cell death nor peroxisome proliferation as the 0.5 g/L exposure concentration has been shown to increase hepatocellular lesions after 100 weeks of treatment without concurrent peroxisome proliferation or cytotoxicity (DeAngelo et al., 1999).

E.2.3.1.5. ***DeAngelo et al., 1989.*** Various strains of rats and mice were exposed to TCA (12 and 31 mM) or DCA (16 and 39 mM) for 14 days with S-D rats and B6C3F1 mice exposed to an additional concentration of 6 mM TCA and 8 mM DCA. Although noting that in a previous study that high concentrations of chloracids, the authors did not measure drinking water consumption in this study. This study exposed several strains of male rats and mice to TCA at two concentrations in drinking water (12 mM and 31mM neutralized TCA) for 14 days. The conversion of mmols/L or mM TCA is 5 g/L TCA, 2 g/L TCA and 1 g/L for 31 mM, 12 mM, and 6 mM TCA, respectively. The conversion of mmols/L of mM DCA is 5 g/L DCA, 2 g/L DCA, and 1 g/L DCA for 39 mM, 16 mM and 8 mM DCA, respectively. The strains of mice tested were Swiss-Webster, B6C3F1, C57BL/6, and C3H and for rats were Sprague Dawley, Osborne Mendel, and F344. For the F344 rat and B6C3F1 mice data from two separate experiments were reported for each. The number of animals in each group was reported to be 6 for most experiments with the exception of the S-D rats ($n = 3$ at the highest dose of TCA and $n = 4$ or 5 for the control and the lower TCA dose), one study in B6C3F1 mice ($n = 4$ or 5 for all groups), and one study in F344 rats ($n = 4$ for all groups). The body weight of the controls was reported to range from 269 to 341 g in the differing strains of rats (1.27-fold) and 21 to 28 g in the differing strains of mice (1.33-fold, age not reported). For percent liver/body weight ratios the range was 4.4 to 5.6% in control rats (1.27-fold) and 5.1 to 6.8% in control mice (1.33-fold).

As discussed in other studies, the determination of PCO activity appears to be highly variable. This enzyme activity is often used as a proxy for peroxisome proliferation. For PCO activity the range of activity in controls was much greater than for either body weight or percent liver/body weight. For rats there was a 2.8-fold difference in PCO control activity and in mice there was a 4.6-fold difference in PCO activity. Between the two studies performed in the same strain of rat (F344) there was a 2.83-fold difference in PCO activity between controls, and for the two studies in the same strain of mouse (B6C3F1) there was a 3.14-fold difference in PCO activity between controls. Not only were there differences between strains and experiments in the same strain, but also differences in control values between species with a wider range of values in the mice. The lowest level of PCO activity in control rats, expressed as nanomoles NAD reduced/min/mg/protein, was 3.34 and for control mice was 1.40. The highest level reported in control in rats was 9.46 and for control mice was 6.40.

These groups of rats and mice were exposed to 2 g/L NaCl, 2 g/L or 5 g/L TCA in drinking water for 14 days and their PCO activity assayed. These doses of TCA did not affect body weight except for the S-D rats, which lost ~16% of their body weight. This was also the same group in which only 3 rats survived treatment. The Osborne-Mendel and F344 strains did not exhibit loss of body weight or mortality due to TCA exposure. There was a large variation in response to TCA exposure between the differing strains of rats and mice with a much larger difference between the strains of mice. For the 3 rat strains tested there was a range between 0% change and 2.38-fold of control for PCO activity at the 5 g/L TCA exposure. For the 2 g/L TCA exposure, there was a range of 0% change to 1.54-fold of control for PCO activity. The Osborne-Mendel rats had 1.54-fold of control value for PCO activity at 2 g/L TCA and 2.38-fold of control value for PCO activity reported at 5 g/L, exhibiting the most consistent increase in PCO with increased dose of TCA. Two experiments were reported for F344 rats with one reporting a 1.63-fold of control and the other a 1.79-fold of control value for 5 g/L TCA. Only one of the F334 experiments also exposed rats to 2 g/L TCA and reported no change from control values.

For the 4 strains of mice tested there was a range of 7.44- to 22.13-fold of control values reported at the 5 g/L TCA exposures and 3.76- to 25.92-fold of control values at the 2 g/L TCA exposures for PCO activity. For the C57BL/6 strain of mice there was little difference between the 5 g/L and 2 g/L TCA exposures and a generally 3-fold higher induction of PCO activity by TCA at the 5 g/L TCA exposure level than for the other mouse strains. Although there was a 2.5-fold difference between the 5 g/L and 2 g/L TCA exposure dose, the difference in magnitude of PCO activity between these doses ranged from 0.85- to 2.23-fold for all strains of mice. For the B6C3F1 mice there was a difference between reported increases of PCO activity in the text (i.e., reported as 9.59-fold of control) for one of the experiments and that presented graphically in Figure 2 (i.e., 8.70-fold of control). Nevertheless in the two studies of B6C3 F1 mice, 5 g/L TCA was reported to induce 7.78-fold of control and 8.70-fold of control for PCO activity, and 2 g/L TCA was reported to induce 5.56-fold of control and 4.70-fold of control for PCO activity. For the two F344 rat studies in which ~200 mg/kg or 5 g/L TCA was administered for 10 or 14 days, there was 1.63-fold of control and 1.79-fold of control values reported for PCO activity. Thus, for experiments in which the same strain and dose of TCA were administered, there was not as large a difference in PCO response than between strains and species.

Whether increases in percent liver/body weight ratios were similar in magnitude to increased PCO activity can be assessed by examination of the differences in magnitude of increase over control for the 5 g/L and 2 g/L TCA treatments in the varying rat strains and mouse strains. The relationship in exposure concentration was a 2.5:1 ratio for the 5 and 2 g/L doses. For rats treatment of 5 g/L TCA to S-D rats resulted in a significant decrease in body weight and therefore, affected the magnitude of increase in percent liver/body weight ratio for this group. However, for the rest of the rat and mouse data, this dose was not reported to affect body weight so that there is more confidence in the dose-response relationship. For the S-D rat there was no change in the percent liver/body weight ratio at 2 g/L but a 10% decrease at 5 g/L TCA exposure with no change in PCO activity for either. However, for the Osborne-Mendel rats, there was no change in percent liver/body weight ratios for either exposure concentration of TCA, but PCO activity was reported to be 1.54-fold of control at 2 g/L and 2.38-fold of control at 5 g/L TCA. Thus, there was a ratio of 2.5-fold increase in PCO activity between the 5 g/L and 2 g/L treatment groups. For the F344 rats there was a 2-fold difference in liver weight increases (i.e., 12 vs. 6% increase over control) between the two exposure concentrations but 1.6-fold of control value for PCO activity at the 5 g/L TCA exposure concentration and no increase in PCO activity at the 2 g/L level. Thus, for the three strains of rats, there did not appear to be a consistent correlation between liver weight induction by TCA and PCO activity.

For differing strains of mice, similar concentrations of TCA were reported to vary in the induction of liver weight increases. The range of liver weight induction was 1.26- to 1.66-fold of control values between the 4 strains of mice at 5 g/L TCA and 1.16- to 1.63-fold at 2 g/L TCA. In general, for mice the magnitudes of the difference in the increase in dose between the 5 g/L and 2 g/TCA exposure concentration (2.5-fold) was generally higher than the increase percent liver/body weight ratios at these doses. The differences in liver weight induction between the 2 and 5 g/L doses were ~40% for the Swiss-Webster, C3H, and for one of the B6C3F1 mouse experiments. For the C57BL/6 mouse there was no difference in liver weight induction between the 2 and 5 g/L TCA exposure groups. For the other B6C3F1 mouse experiments there was a 2.5-fold greater induction of liver weight increase for the 5 g/L TCA group than for the 2 g/L exposure group (1.39-fold of control vs. 1.16-fold of control for percent liver/body weight, respectively). For PCO activity the Swiss-Webster, C3H, and one of the B6C3F1 mouse experiments were reported to have ~2-fold difference in the increase in PCO activity between the two doses. For the other B6C3F1 mouse experiment there was only about a 50% increase and for the C57BL/6 mouse data there was 15% less PCO activity induction reported at the 5 g/L TCA dose that at the 2 g/L dose. None of the difference in increases in liver weight or PCO activity in mice from the 2 or 5 g/L TCA exposures were of the same magnitude as the difference in TCA exposure concentration (i.e., 2.5-fold) except for liver weight from the one experiment in B6C3F1 mice. This is also the data used fore comparisons with the Sprague-Dawley rat discussed below.

In regard to strain differences for TCA response in mice, there did not appear to be correlations of the magnitude of 5 g/L TCA-induced changes in percent liver/body weight ratio or PCO activity, with the body weights reported for control mice for each strain. The control weights between the 4 strains of mice varied from 21 to 28 grams. The strain with the greatest response (C57Bl/6) for TCA-induced changes in percent liver/body weight ratio (i.e., 1.66-fold of control) and PCO activity (22.13-fold of control) had a mean body weight reported to be 26 g for controls. At this dose, the range of percent liver/body weight for the other strains was reported to be 1.26- to 1.39-fold of control and the range of PCO activity reported to be of 7.48- to 8.71-fold of control.

Of note is that in the literature, this study has been cited as providing evidence of differences between rats and mice for peroxisomal response to TCA and DCA. Generally the PCO data from the Sprague Dawley rats and B6C3F1 mice at the highest dose of TCA and DCA have been cited. However, the S-D strain was reported to have greater mortality from TCA at this exposure than the other strains tested (i.e., only 3 rats survived and provided PCO levels) and a lower PCO response (no change in PCO activity over control) that the other two strains tested in this study (i.e., Osborne-Mendel rats was reported to have had 2.38-fold of control and the F344-had a 1.63- to 1.79-fold of control for PCO activity after exposure to 5 g/L TCA with no mortality). The B6C3F1 mouse was reported to have a 7.78- or 8.71-fold of control for PCO activity from 5 g/L TCA exposure. Certainly the male mouse is more responsive to TCA induction of PCO activity. However, as discussed above there are large variations in control levels of PCO activity and in the magnitude and dose-response of TCA-induction of PCO activity between rat and mouse strains and between species. If is not correct to state that the rat is refractory to TCA-induction of peroxisome activity.

Unfortunately, the authors chose the S-D rat (i.e., the most unresponsive strain for PCO activity and most sensitive to toxicity) for studies for comparative studies between DCA and TCA effects. The authors also tested for carnitine acetyl CoA transferase (CAT) activity as a marker of peroxisomal enzyme response and took morphometric analysis of peroxisome # and cytoplasmic volume for one liver section for each of two B6C3F1 mice of S-D rats from the 5 g/L TCA and 5 g/L DCA treatment groups. Only 6 electron micrograph fields were analyzed from each section (12 fields total) were analyzed without identification as to what area of the liver lobules they were being taken from. Hence there is a question as to whether the areas which are known to be peroxisome rich were assayed of not. Also as noted above, previous studies have indicate that such high concentration of DCA and TCA inhibit drinking water consumption and therefore, raising issues not only about toxicity but also the dose which rats and mice received. The number of peroxisomes per 100 μm^3 and cytoplasmic volume of peroxisomes was reported to be 6.60 and 1.94%, respectively, for control rats, and 6.89 and 0.61% for control mice, respectively. For 5 g/L TCA and 5 g/L DCA the numbers of peroxisomes were reported to be increased to 7.14 and 16.75, respectively in treated Sprague Dawley rats. Thus, there was 2.5- and 1.08-fold of control reported in peroxisome # for 5 g/L DCA and TCA, respectively. The cytoplasmic volume of peroxisomes was reported to be 2.80% and 0.89% for 5 g/L DCA and 5 g/L TCA, respectively (i.e., a 1.44-fold of control and ~60% reduction for 5 g/L DCA and 5 g/L TCA, respectively). Thus, 5 g/L TCA was reported to slightly increase the number of peroxisomes and but decrease the percent of the cytoplasmic volume occupied by peroxisome by half. For DCA the reported pattern was for both to increase. PCO activity was reported to increase by a similar magnitude as peroxisome # but not volume in the 5 g/L TCA treated S-D rats. However, although peroxisomal volume was reported to be cut nearly in half and for peroxisome number to be similar, 5 g/L TCA treatment was not reported to change PCO activity in the S-D rat.

For comparisons between DCA and TCA B6C3 F1 mice were examined at 1.0, 2.0, and 5.0 g/L concentrations. DCA was reported to induce a higher percent liver/body weight ratio that did TCA at every concentration (i.e., 1.55-, 1.27-, and 1.21-fold of control for DCA and 1.39-, 1.16-, and 1.08-fold of control for TCA at 1.0, 2.0, and 5.0 g/L concentrations, respectively). As noted above, for other strains of mice tested and a second experiment with B6C3F1 mice, there was 40% or less difference in percent liver/body weight ratio between the 2.0 g/L and 5.0 g/L exposures to TCA but for this experiment there was a 2.5-fold difference. Thus, at 5 g/L there was ~40% greater induction of liver weight for DCA than TCA. In the B6C3F1 mice, 5 g/L TCA was reported to increase peroxisome number to 30.75 and cytoplasmic volume to 4.92% (i.e., 4.4- and 8.1-fold of control, respectively). For 5 g/L DCA treatment, the peroxisome number was reported to be 30.77 and 3.75% (i.e., 4.5- and 6.1-fold of control, respectively). While there was no difference in peroxisome number and ~40% difference in cytoplasmic volume at the 5.0 g/L exposures of DCA and TCA, there was a greater difference in the magnitude of PCO activity increase. The 5 g/L TCA exposure was reported to induce 4.3-fold of control for PCO activity while 5 g/L DCA induced as 9.6-fold of control PCO activity (although a figure in the report shows 8.7-fold of control) which is a ~2.5-fold difference between DCA and TCA at this exposure concentration. Thus, for one of the B6C3F1 mouse studies, 5 g/L DCA and TCA treatments were reported to give a similar increase peroxisome number, TCA to induce a 40% greater increase in peroxisomal cytoplasmic volume than DCA and a 2.5-fold greater increase in PCO activity, but DCA to induce ~40% greater liver weight induction than TCA.

Not only were PCO activity, peroxisome number and cytoplasmic volume occupied by peroxisomes analyzed but also CAT activity as a measure of peroxisome proliferation. For TCA and DCA the results were opposite those reported for PCO activity. In S-D rats control levels of CAT were reported to be 1.81 nmoles of carnitine transferred/min/mg/protein. Exposure to 5 g/L TCA was reported to increase CAT activity by 3.21-fold of control while 5 g/L DCA was reported to induce CAT activity to 10.33-fold of control levels in S-D rats. However, while PCO activity was reported to be the same as controls, and peroxisomal volume decreased, 5 g/L TCA increased CAT activity 3.21-fold of control in these rats. The level of CAT induced by 5 g/L DCA was over 10-fold of control in the rat while peroxisome # was only 2.5-fold of control and cytoplasmic volume 1.4-fold of control. Thus, the fold increases for these three measures were not the same for DCA treatment and for TCA in rats. Nevertheless for CAT, DCA was a stronger inducer in rats than was TCA. In B6C3 F1 mice 5 g/L TCA and 5 g/L DCA induced CAT activity to a similar extent (4.50- and 5.61-fold of control, respectively). The magnitude of CAT induction was similar to that of peroxisome # for both 5 g/L DCA and 5 g/L TCA and lower than PCO activity in DCA-treated mice and cytoplasmic volume in TCA-treated mice by about half. Thus, using CAT as the marker of peroxisome proliferation, the rat was more responsive than the mouse to DCA and nearly as responsive to TCA as the mouse at this high dose in these two specific strains. These data illustrate the difficulty of using only one measure for peroxisome proliferation and shows that the magnitude of increased PCO activity is not necessarily predictive of the peroxisome # or cytoplasmic volume or CAT activity. The difficulty of interpretation of the data from so few animals and sections for the electron microscopy analysis, and the low number of animals for PCO activity and CAT activity (n = 3 to 6), the high dose studied (5 g/L), and the selection of a rat strain that appears to be more resistant to this activity but more susceptible to toxicity than the others tested, should be taken into account before conclusions can be made about differences between these chemicals for peroxisome activity between species.

Of note is that PCO activity was also shown to be increased by corn oil alone in F344 rats and to potentiate the induction of PCO activity of TCA. After 10 days of exposure to either water, corn oil, 200 mg/kg/d TCA in corn oil or 200 mg/kg TCA in water via gavage dosing, there was 1.40-fold PCO activity from corn oil treatment alone in comparison to water, a 1.79-fold PCO activity from TCA in water treatment in comparison to water, and a 3.14-fold PCO activity from TCA in corn oil treatment in comparison to water.

The authors provided data for 3 concentrations of DCA and TCA for S-D and for one experiment in the B6C3F1 mouse for examination of changes in body and percent liver/body weight ratios (1, 2, or 5 g/L DCA or TCA) after 14 days of exposure. As noted above, not only did the 5 g/L exposure concentration of DCA result in mortality in the S-D strain of rat, but the 5 g/L and 2 g/L concentrations of DCA were reported to decrease body weight (~20 and 25%, respectively). The 5 g/L dose of TCA was also reported to induce a statistically significant decrease in body weight in the S-D rat. There were no differences in final body weight in any of the mice exposed to TCA or DCA. As noted above no TCA or DCA exposure group of S-D rats was reported to have a statistically significant increase in percent liver/body weight ratio over control. For the B6C3F1 male mice, the percent liver/body weight ratio was 1.22-, 1.27-, and 1.55-fold of control after exposure to 1, 2, and 5 g/L DCA, respectively, and 1.08-, 1.16-, and 1.39-fold of control after exposure to 1, 2, and 5 g/L TCA, respectively. Thus, for DCA there was only a 20% increase in liver weight corresponding to the 2-fold increase between the 1 and 2 g/L exposure levels of DCA. Between the 2 and 5 g/L exposure concentrations of DCA there was a 2-fold increase in liver weight corresponding to a 2.5-fold increase in exposure concentration. For TCA, the magnitude of increase in dose was reported to be proportional to the magnitude of increase in percent liver/body weight ratio in the B6C3 F1 male mouse. As stated above, the correspondence between magnitude of dose and percent liver weight for TCA exposure in this experiment differed from the other experiment reported for this strain of mouse and also differed from the other 3 strains of mice examined in this study where the magnitude in liver weight gain was much less than exposure concentration.

E.2.3.2. *Subchronic and Chronic Studies of Dichloroacetic Acid (DCA) and Trichloroacetic Acid (TCA)*

Several experiments have been conducted with exposure to DCA and TCA, generally at very high levels with a limited dose range, for less periods of time than standard carcinogenicity bioassays, and with very limited information on any endpoints other than the liver tumor induction. Caldwell and Keshava (2006) and Caldwell et al. (2008b) have examined these studies for inferences of modes of action for TCE. Key studies are briefly described below for comparative purposes of results reported in TCE studies.

E.2.3.2.1. ***Snyder et al., 1995.*** Studies of TCE have reported either no change or a slight increase in apoptosis only after a relatively high exposure level (Dees and Travis, 1993; Channel et al., 1998). Inhibition of apoptosis, which has been suggested to prevent removal of "initiated" cells from the liver and lead to increased survival of precancerous cells, has been proposed as part of the MOA for peroxisome proliferators (see Section E.3.4). The focus of this study was to examine whether DCA, which has been shown to inhibit DNA synthesis after an initial transient increase (see Section E.2.3.3, below), also alters the frequency of spontaneous apoptosis in mice. This study exposed 28-day old male B6C3F1 male mice (n = 5) to 0, 0.5 or 5.0 g/L buffered DCA in drinking water for up to 30 days (Phase I = 5−15 days exposure and Phase II = 20−30 days treatment). Portions of the left lobe of the liver were prepared for histological examination after H&E staining. Hepatocyte number was determined by counting nuclei in 50 fields with nonparenchymal cell nuclei excluded on the basis of nuclear size. Multinucleate cells were counted as one cell. Apoptotic cells were visualized by in situ TDT nick end-labeling assay from 2−4 different liver sections from each control or treated animal. The average number of apoptotic cells was then determined for each animal in each group. The authors reported that in none of the tissues examined were necrotic foci observed, there was no any indication of lymphocyte or neutrophil infiltration indicative of an inflammatory response, and suggested that no necrotic cells contributed to the responses in their analysis.

Control animals were reported to exhibit apoptotic frequencies ranging from ~0.04 to 0.085% and that over the 30-day period the frequency rate declined. The authors suggested that this result is consistent with reports of the livers of these young animals undergoing rapid changes in cell death and proliferation. They note that animals receiving 0.5 g/L DCA also had a similar trend of decreasing apoptosis with age, supportive of the decrease being a physiological phenomenon. The 0.5 g/L exposure level of DCA was reported to decrease the percentage of apoptotic hepatocytes as the earliest time point studied and to remain statistically significantly decreased from controls from 5 to 30 days of exposure. The rate of apoptosis ranged from ~0.025 to 0.060% after 0.5 g/L DCA exposure during the 30-day period (i.e., and ~30−40% reduction). Animals receiving the 5.0 g/L DCA dose exhibited a significant reduction at the earliest time point that was sustained at a similar level and statistically significant throughout the time-course of the experiment (percent apoptosis ranged from 0.015−0.030%). The results of this study not only provides a baseline of apoptosis in the mouse liver, which is very low, but also to show the importance of taking into account the effects of age on such determinations. The authors reported that the for rat liver the estimated frequency of spontaneous apoptosis to be ~0.1% and therefore, greater than that of the mouse. The significance of the DCA-induced reduction in apoptosis, of a level that is already inherently low in the mouse, for the MOA for induction of cancer is difficult to discern.

E.2.3.2.2. ***Mather et al., 1990.*** This 90-day study in male S-D rats examined the body and organ weight changes, liver enzyme levels, and PCO activity in livers from rats treated with estimated concentrations of 3.9, 35.5, 345 mg/kg day DCA or 4.1, 36.5, or 355 mg/kg/d TCA from drinking water exposures (i.e., 0, 50, 500 and 5,000 ppm or 0.05, 0.5, or 5.0 g/L DCA or TCA in the drinking water). All dose levels of DCA and TCA were reported to result in a dose-dependent decrease in fluid intake at 2 months of exposure. The rats were 9 (DCA) or 10 (TCA) weeks old at the beginning of the study (n = 10/group). Animals with body weights that varied more than 20% of mean weights were discarded from the study. The DCA and TCA solutions were neutralized. The mean values for initial weights of the animals in each test group varied less than 3%. DCA treatment induced a dose-related decrease in body weight that was statistically significant at the two highest levels (i.e., a 6, 9.5, and 17% decrease from control). TCA treatment also resulted in lower body weights that were not statistically significant (i.e., 2.1, 4.4, and 5.9%). DCA treatments were reported to result in a dose-related increase in absolute liver weights (1.01-, 1.13-, and 1.36-fold of control that were significantly different at the highest level) and percent liver/body weight ratios (1.07-, 1.24-, and 1.69-fold of control that were significant at the two highest dose levels). TCA treatments were reported to not result in changes in either absolute liver weights or percent liver/body weight ratios with the exception of statistically significant increase in percent liver/body weight ratios at the highest level of treatment (1.02-fold of control). Total serum protein levels were reported to be significantly depressed in all animals treated with DCA with animals in the two highest dose groups also exhibiting elevations of alkaline phosphatase. Alanine-amino transferase levels were reported to be elevated only in the highest treatment group. No consistent treatment-related effect on serum chemistry was reported to be observed for the TCA-treated animals with data not shown. In terms of PCO activity, there was only a mild increase at the highest dose of 15% for TCA and a 2.5-fold level of control for DCA treatment that were statistically significant. The difference in PCO activity between control groups for the DCA and TCA experiments was reported to be 33%. No treatment affect was reported to be apparent for hepatic microsomal enzymes, or measures of immunotoxicity for either DCA or TCA but data were not shown. Focal areas of hepatocellular enlargement in both DCA- and TCA-treated rats were reported to be present with intracellular swelling more severe with the highest dose of DCA treatment. Livers from DCA treated rats were reported to stain positively for PAS, indicating significant amounts of glycogen with TCA treated rats reported to display "less evidence of glycogen accumulation." Of note is that, in this study of rats, DCA was reported to induce a greater level of PCO activity than did TCA.

E.2.3.2.3. ***Parrish et al., 1996.*** Parrish et al. (1996) exposed male B6C3F1 mice (8 weeks old and 20−22 g upon purchase) to TCA or DCA (0, 0.01, 0.5, and 2.0 g/L) for 3 or 10 weeks (n = 6). Livers were excised and nuclei isolated for examination of 8-OHdG and homogenates examined for cyanide insensitive acyl-CoA oxidase (ACO) and laurate hydroxylase activity. The authors noted that control values between experiments varied as much as a factor of 2-fold for PCO activity and that data were presented as percent of concurrent controls. Initial body weights for treatment groups were not presented and thus, differences in mean values between the groups cannot be ascertained.

Final body weights were reported to not be statistically significantly changed by DCA or TCA treatments at 21 days or 71 days of treatment (all were within ~8% of controls). The mean percent liver/body ratios were reported to be 5.4, 5.3, 6.1, and 7.2% for control, 0.1, 0.5, and 2.0 g/L TCA, respectively and 5.4, 5.5, 6.7, and 7.9% for control, 0.1, 0.5, and 2.0 g/L DCA, respectively after 21 days of exposure. This represents 0.98-, 1.13-, and 1.33-fold of control levels with these exposure levels of TCA and 1.02-, 1.24-, and 1.46-fold of control levels with DCA after 21 days of exposure. For 71 days of exposure the mean percent liver/body ratios were reported to be 5.1, 4.6, 5.8, and 6.9% for control, 0.1, 0.5, and 2.0 g/L TCA, respectively and 5.1, 5.1, 5.9, and 8.5% for control, 0.1, 0.5, and 2.0 g/L DCA, respectively. This represents 0.90-, 1.14-, and 1.35-fold of control with TCA exposure and 1.0-, 1.15-, and 1.67-fold of control with DCA exposure after 71 days of exposure. The magnitude of difference between the 0.1 and

0.5 g/L TCA doses is 5 and 0.5 and 2.0 g/L doses is 4-fold. For the 21-day and 71-day exposures the magnitudes of the increases in percent liver/body weight over control values were greater for DCA than TCA exposure at same concentration with the exception of 0.5 g/L doses at 71 days in which both TCA and DCA induced similar increases. For TCA, the 0.01 g/L dose produces a similar 10% decrease in percent liver/body weight. Although there was a 4-fold increase in magnitude between the 0.5 and 2.0 g/L TCA exposure concentrations, the magnitude of increase for percent liver/body weight increase was 2.5-fold between them at both 21 and 71 days of exposure. For DCA, the 0.1 g/L dose was reported to have a similar value as control for percent liver/body weight ratio. Although there was a 4-fold difference in dose between the 0.5 and 2.0 g/L DCA exposure concentrations, there was a ~2-fold increase in percent liver/body weight increase at 21 days and ~4.5-fold increase at 71 days.

As a percentage of control values, TCA was reported to induce a dose-related increase in PCO activity at 21 days (~1.5-, 2.2-, and ~4.1-fold of control, for 0.1, 0.5, and 2 g/L TCA exposures). Only the 2.0 g/L dose of DCA was reported to induce a statistically significant increase at 21-days of exposure of PCO activity over control (~1.8-fold of control) with the 0.1 and 0.5 g/L exposure PCO activity to be slightly less than control values (~20% less). Thus, although there was no increase in percent liver/body weight at 0.1 g/L TCA, the PCO activity was reported to be increased by ~50% after 21 days. A 13% increase in liver weight at 0.5 g/L TCA was reported to be associated with 2.2-fold of control level of PCO activity and a 33% increase in liver weight after 2.0 g/L TCA to be associated with 4.1-fold of control level of PCO activity. Thus, increases in PCO activity were not necessarily correlated with concurrent TCA-induced increases in liver weight and the magnitudes of increase in liver weight between 0.5 and 2.0 g/L TCA (2.5-fold) was greater than the corresponding increase in PCO activity (1.8-fold of control). Although there was a 20-fold difference in TCA dose, the magnitude of increase in PCO activity between 0.1 and 2.0 g/L TCA was ~2.7-fold. As stated above, the 4-fold difference in TCA dose at the two highest levels resulted in a 2.5-fold increase in liver weight. For DCA, the increases in liver weight at 0.1 and 0.5 g/L DCA exposures were not associated with increased PCO activity after 21 days of exposure. The 2.0 g/L DCA exposure concentration was reported to induce 1.8-fold of control PCO activity. After 71 days of treatment, TCA induced a dose-related increase in PCO activity that was approximately twice the magnitude as that reported at 21 days (i.e., ~9-fold greater at 2.0 g/L level). After 71 days, for DCA the 0.1 and 0.5 g/L doses produced a statistically significant increase in PCO activity (~1.5- and 2.5-fold of control, respectively). The administration of 1.25 g/L clofibric acid in drinking water was used as a positive control and reported to induce ~6−7-fold of control PCO activity at 21 and 71 days of exposure.

Laurate hydroxylase activity was reported to be elevated significantly only by TCA at 21 days (2.0 g/L TCA dose only) and to increased to approximately the same extent (~1.4 to 1.6-fold of control values) at all doses tested. For 0.1 g/L DCA the laurate hydroxylase activity was reported to be similar to that of 0.1 g/L TCA (~1.4-fold of control) but to be ~1.2-fold of control at both the 0.5 and 2.0 g/L DCA exposures. At 71 days, both the 0.5 and 2.0 g/L TCA exposures induced a statistically significant increase in laurate hydroxylase activity (i.e., 1.6- and 2.5-fold of control, respectively) with no change after DCA exposure. The actual data rather than percent of control values were reported for laurate hydroxylase activity. The control values for laurate hydroxylase activity varied 1.7-fold between 21 and 71 days experiments. The results for 8-OHdG levels are discussed in Section E.3.4.2.3, below. Of note is that the increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged, see Section E.3.4.2.3, below) and also not with changes laurate hydrolase activity or percent liver/body weight ratio increases observed after either DCA or TCA exposure. A strength of this study is that is examined exposure concentrations that were lower than those examined in many other short-term studies of DCA and TCA.

E.2.3.2.4. ***Bull et al., 1990.*** The focus of this study was the determination of “dose-response relationships in the tumorigenic response to these chemicals [sic DCA and TCA] in B6C3F1 mice, determine the nature of the nontumor pathology that results from the administration of these compounds in drinking water, and test the reversibility of the response.” Male and female B6C3F1 mice (age 37 days) were treated from 15 to 52 weeks with neutralized TCA and TCA. A highly variable number and generally low number of animals were reported to be examined in the study with $n = 5$ for all time periods except for 52 weeks where in males the $n = 35$ for controls, $n = 11$ for 1 g/L DCA, $n = 24$ for 2 g/L DCA, $n = 11$ for 1 g/L TCA, and $n = 24$ for 2 g/L TCA exposed mice. Female mice were only examined after 52 weeks of exposure and the number of animals examined was $n = 10$ for control, 2 g/L DCA, and 2 g/L TCA exposed mice. “Lesions to be examined histologically for pathological examination were selected by a random process” with lesions reported to be selected from 31 of 65 animals with lesions at necropsy. 73 of 165 lesions identified in 41 animals were reported to be examined histologically. All hyperplastic nodules, adenomas and carcinomas were lumped together and characterized as hepatoproliferative lesions. Accordingly there were only exposure concentrations available for dose-response analyses in males and only “multiplicity of hepatoproliferative lesions” were reported from random samples. Thus, these data cannot be compared to other studies and are unsuitable for dose-response with inadequate analysis performed on random samples for pathological examination. The authors state that some of the lesions taken at necropsy and assumed to be proliferative were actually histologically normal, necrotic, or an abscess as well. It is also limited by a relatively small number of animals examined in regard to adequate statistical power to determine quantitative differences. Similar concerns were raised by Caldwell et al. (2008b) with a subsequent study (e.g., Bull et al., 2002). For example, the authors report that 5/11 animals had "lesions" at 1 g/L TCA at 52 weeks and 19/24 animals had lesions at 2 g/L TCA at 52 weeks. However, while 7 lesions were examined in 5 mice bearing lesions at 1 g/L TCA, only 16 of 30 lesions from 11 of the 19 animals bearing lesions examined in the 2 g/L TCA group. Therefore, almost half of the mice with lesions were not examined histologically in that group along with only half of the "lesions."

The authors reported the effects of DCA and TCA exposure on liver weight and percent liver/body changes ($m \pm$ SEM) and these results gave a pattern of hepatomegaly generally consistent with short-term exposure studies. The authors report "no treatment produced significant changes in the body weight or kidney weight of the animals (data not shown)" In male mice ($n = 5$) at 37 weeks of exposure, liver weights were reported to be 1.6 ± 0.1, 2.5 ± 0.1, and 1.9 ± 0.1 g for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. The percent liver/body weights were reported to be 4.1% ± 0.3%, 7.3% ± 0.2%, and 5.1% ± 0.1% for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. In male mice at 52 weeks of exposure, liver weights were reported to be 1.7 ± 0.1, 2.5 ± 0.1, 5.1 ± 0.1, 2.2 ± 0.1, and 2.7 ± 0.1 g for control ($n = 35$), 1 g/L DCA ($n = 11$), 2 g/L DCA ($n = 24$), 1 g/L TCA ($n = 11$), and 2 g/L TCA ($n = 24$) exposed mice, respectively. In male mice at 52 weeks of exposure, percent liver/body weights were reported to be 4.6% ± 0.1%, 6.5% ± 0.2%, 10.5% ± 0.4%, 6.0% ± 0.3%, and 7.5% ± 0.5% for control, 1 g/L DCA, 2 g/L DCA, 1 g/L TCA, and 2 g/L TCA exposed mice, respectively. For female mice ($n = 10$) at 52 weeks of exposure, liver weights were reported to be 1.3 ± 0.1, 2.6 ± 0.1, and 1.7 ± 0.1 g for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. The percent liver/body weights were reported to be 4.8% ± 0.3%, 9.0% ± 0.2%, and 6.0% ± 0.3% for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. Although the number of animals examined varied 3-fold between treatment groups in male mice, the authors reported that all DCA and TCA treatments were statistically increased over control values for liver weight and percent body/liver weight in both genders of mice. In terms of percent liver/body weight ratio, female mice appeared to be as responsive as males at the exposure concentration tested. Thus, hepatomegaly reported at these exposure levels after short-term exposures appeared to be further increased by chronic exposure with equivalent levels of DCA inducing greater hepatomegaly than TCA.

Interestingly, after 37 weeks of treatment and then a cessation of exposure for 15 weeks liver weights were assessed in control male mice, 2 g/L DCA treated mice, and 2 g/L TCA treated mice (n = 11 for each group but results for controls were pooled and therefore, n = 35). Liver weights were reported to be 1.7 ± 0.1, 2.2 ± 0.1, and 1.9 ± 0.1 g for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. The percent liver/body weights were reported to be 4.6% ± 0.1%, 5.7% ± 0.3%, and 5.4% ± 0.2% for control, 2 g/L DCA, and 2 g/L TCA exposed mice, respectively. After 15 weeks of cessation of exposure, liver weight and percent liver/body weight were reported to still be statistically significantly elevated after DCA or TCA treatment. The authors partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. The authors state that because of the low incidence of lesions in the control group and the two groups that had treatments suspended, all the lesions from these groups were included for histological sectioning. However, the authors present a table indicating that, of the 23 lesions detected in 7 mice exposed to DCA for 37 weeks, 19 were examined histologically. Therefore, groups that were exposed for 52 weeks had a different procedure for tissue examination as those at 37 weeks. In terms of liver tumor induction, the authors stated that "statistical analysis of tumor incidence employed a general linear model ANOVA with contrasts for linearity and deviations from linearity to determine if results from groups in which treatments were discontinued after 37 weeks were lower than would have been predicted by the total dose consumed." The multiplicity of tumors observed in male mice exposed to DCA or TCA at 37 weeks and then sacrificed at 52 weeks were reported by the authors to have a response in animals that received DCA very close to that which would be predicted from the total dose consumed by these animals. The response to TCA was reported by the authors to deviate significantly (p = 0.022) from the linear model predicted by the total dose consumed. Multiplicity of lesions per mouse and not incidence was used as the measure. Most importantly the data used to predict the dose response for "lesions" used a different methodology at 52 weeks than those at 37 weeks. Not only were not all animal's lesions examined but foci, adenomas, and carcinomas were combined into one measure. Therefore, foci, of which a certain percentage have been commonly shown to spontaneously regress with time, were included in the calculation of total "lesions." Pereira and Phelps (1996) note that in initiated mice treated with DCA, the yield of altered hepatocytes decreases as the tumor yields increase between 31 and 51 weeks of exposure suggesting progression of foci to adenomas. Initiated and noninitiated control mice also had fewer foci/mouse with time. Because of differences in methodology and the lack of discernment between foci, adenomas, and carcinomas for many of the mice exposed for 52 weeks, it is difficult to compare differences in composition of the "lesions" after cessation of exposure. For TCA treatment the number of animals examined for determination of which "lesions" were foci, adenomas, and carcinomas was 11 out of the 19 mice with "lesions" at 52 weeks while all 4 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. For DCA treatment the number of animals examined was only 10 out of 23 mice with "lesions" at 52 weeks while all 7 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. Most importantly, when lesions were examined microscopically then did not all turn out to be preneoplastic or neoplastic. Two lesions appeared "to be histologically normal" and one necrotic. Not only were a smaller number of animals examined for the cessation exposure than continuous exposure but only the 2 g/L exposure levels of DCA and TCA were studied for cessation. The number of animals bearing "lesions" at 37 and then 15 week cessation weeks was 7/11 (64%) while the number of animals bearing lesions at 5 weeks was 23/24 (96%) after 2 g/L DCA exposure. For TCA the number of animals bearing lesions at 37 weeks and then 15 weeks cessation was 4/11 (35%) while the number of animals bearing lesions at 52 weeks was 19/24 (80%). While suggesting that cessation of exposure diminished the number of "lesions," conclusions regarding the identity and progression of those lesion with continuous versus noncontinuous DCA and TCA treatment are tenuous.

Macroscopically, the "livers of many mice receiving DCA in their drinking water displayed light colored streaks on the surface" at every sacrifice period and "corresponded with multi-focal areas of necrosis with frequent infiltration of lymphocytes." At the light microscopic level, the lesions were described to also be present in the interior of the liver as well. For TCA-treated mice, "similar necrotic lesions were also observed… but at a much lower frequency, making it difficult to determine if they were treatment-related." Control animals were reported not to show degenerative changes. "Marked cytomegaly" was reported for mice treated with either 1 or 2 g/L DCA "throughout the liver" In regard to cell size the authors did not give any description in the methods section of the paper as to how sections were selected for morphometric analysis or what areas of the liver acinus were examined but reported after 52 weeks of treatment the long axis of hepatocytes measured (mean ± S.E.) 24.9 ± 0.3, 38.5 ± 1.0, and 29.3 ± 1.4 μm in control, DCA- and TCA-treated mice, respectively.

Mice treated with TCA (2 g/L) for 52 weeks were reported to have livers with "considerable dose-related accumulations of lipofuscin." However, no quantitative analyses were presented. A series of figures representative of treatment showed photographs (1,000×) of lipofuscin fluorescence indicating greater fluorescence in TCA treated liver than control or DCA treated liver.

A series of photographs of H&E sections in the report (see Figures 2a, b and c) are shown as representative histology of control mice, mice treated with 2 g/L DCA and 2 g/L TCA. The area of the liver from which the photographs were taken did not include either portal tract or central veins and the authors did not give the zone of the livers from which they were taken. The figure representing TCA treatment shows only a mild increase in cell volume in comparison to controls, while for DCA treatment the hepatocyte diameter was greatly enlarged, pale stained so that cytoplasmic contents appear absent, nuclei often pushed to the cell perimeter, and the sinusoids appearing to be obscured by the swollen hepatocytes. The apparent reduction of sinusoidal volume by the enlarged hepatocytes raises the possibility of decreased blood flow through the liver, which may have been linked to focal areas of necrosis reported for this high exposure level. In a second set of figures, glycogen accumulation was shown with PAS staining at the same level of power (400×) for the same animals. In control animals PAS positive material was not uniformly distributed between or within hepatocytes but send to show a zonal pattern of moderate intensity. PAS positive staining (which the authors reported to be glycogen) appeared to be slightly less than controls but with a similar pattern in the photograph representing TCA exposure. However, for DCA the photograph showed a uniform and heavy stain within each hepatocyte and across all hepatocytes. The authors stated in the results section of the paper that "the livers of TCA-treated animals displayed less evidence of glycogen accumulation and it was more prominent in periportal than centrilobular portions of the liver acinus." In their abstract they state "TCA produced small increases in cell size and a much more modest accumulation of glycogen." Thus, the statement in the text, which is suggestive that TCA induced an increase in glycogen over controls that was not as much as that induced by DCA, and the statement in the abstract which concludes TCA exposure increased glycogen is not consistent with the photographs. In the photograph shown for TCA there is less not more PAS positive staining associated with TCA treatment in comparison to controls. In Sanchez and Bull (1990) the authors report that "TCA exposure induced a much less intense level of PAS staining that was confined to periportal areas" but do not compare PAS staining to controls but only to DCA treatment. In the discussion section of the paper the authors state "Except for a small increase in liver weight and cell size, the effects produced by DCA were not observed with TCA." Thus, there seems to be a discrepancy with regard to what the effects of TCA are in relation to control animals from this report that has caused confusion in the literature. Kato-Weinstein et al. (2001) reported that in male mice exposed to DCA and TCA the DCA increased glycogen and TCA decreased glycogen content of the liver using chemical measurement of glycogen in liver homogenates and using ethanol-fixed sections stained with PAS, a procedure designed to minimize glycogen loss.

E.2.3.2.5. ***Nelson et al., 1990.*** Nelson et al. (1990) reported that they used the same exposure paradigm as Herren-Freund et al. (1987), with little description of methods used in treatment of the animals. Male B6C3F1 mice were reported to be exposed to DCA (1 or 2 g/L) or TCA (1 or 2 g/L) for 52 weeks. The number of animals examined for nontumor tissue was 12 for controls.

The number of animals varied from 2 to 8 for examination of nontumor tissue, hyperplastic nodules, and carcinoma tissues for c-Myc expression. There was no description for how hyperplastic nodules were defined and whether they included adenomas and foci. For the 52-week experiments, the results were pooled for lesions that had been obtained by exposure to the higher or lower concentrations of DCA or TCA (i.e., the TCA results are for lesions induced by either 1.0 g/L or 2.0 g/L TCA). A second group of mice were reported to be given either DCA or TCA for 37 weeks and then normal drinking water for the remaining time till 52 weeks with no concentrations given for the exposures to these animals. Therefore, it is impossible to discern what dose was used for tumors analyzed for c-Myc expression in the 37-week treatment groups and if the same dose was used for 37 and 52 week results. Autoradiography was described for 3 different sections per animal in 5 different randomly chosen high power fields per section. The number of hyperplastic nodules or the number of carcinomas per animal induced by these treatments was not reported nor the criteria for selection of lesions for c-myc expression. Apparently a second experiment was performed to determine the expression of c-H-ras. Whereas in the first experiment there were no hyperplastic nodules, in the second 1-control animal was reported to have a hyperplastic nodule. The number of control animals reported to be examined for nontumor tissue in the second group was 12. The numbers of animals in the second group was reported to vary from 1 to 7 for examination of nontumor tissue, hyperplastic nodules, and carcinoma tissues for c-H-ras expression. The number of animals per group for the investigation of H-ras did not match the numbers reported for that of c-Myc. The number of animals treated to obtain the "lesion" results was not presented (i.e., how many animals were tested to get a specific number of animals with tumors that were then examined). The number of lesions assessed per animal was not reported.

At 52 weeks of exposure, hyperplastic nodules (n = 8 animals) and carcinomas (n = 6 animals) were reported to have ~2-fold expression of c-Myc relative to nontumor tissue (n = 6 animals) after DCA treatment. After 37 weeks of DCA treatment and cessation of exposure, there was a ~30% increase in c-Myc in hyperplastic nodules (n = 4 animals) that was not statistically significant. There were no carcinomas reported at this time. After 52 weeks of TCA exposure, there was ~2-fold of nontumor tissue reported for c-Myc in hyperplastic nodules (n = 6 animals) and ~3-fold reported for carcinomas (n = 6 animals). After 37 weeks of TCA exposure there was ~2-fold c-Myc in hyperplastic nodules (n = 2 animals) that was not statistically significant and ~2.6-fold increase in carcinomas (n = 3 animals) that was reported to be statistically significant over nontumor tissue. There was no difference in c-Myc expression between untreated animals and nontumor tissue in the treated animals.

The authors reported that c-Myc expression in TCA-induced carcinomas was "almost 6 times that in control tissue (corrected by subtracting nonspecific binding)," and concluded that c-Myc in TCA-induced carcinomas was significantly greater than in hyperplastic nodules or carcinomas and hyperplastic nodules induced by DCA. However, the c-myc expression reported as the number of grains per cells was ~2.6-fold in TCA-induced carcinomas and ~2-fold in DCA-induced carcinomas than control or nontumor tissue at 52 weeks. The hyperplastic nodules from DCA- and TCA-treatments at 52 weeks gave identical ratios of ~2-fold. In 3 animals per treatment, c-Myc expression was reported to be similar in "selected areas of high expression" for either DCA or TCA treatments of 52 weeks.

There did not appear to be a difference in c-H-ras expression between control and nontumor tissue from DCA- or TCA-treated mice. The levels of c-H-ras transcripts were reported to be "slightly elevated" in hyperplastic nodules induced by DCA (~67%) or TCA (~43%) but these elevations were not statistically significant in comparison to controls. However, carcinomas "derived from either DCA- or TCA-treated animals were reported to have significantly increased c-H-ras levels relative to controls." The fold increase of nontumor tissue at 52 weeks for DCA-induced carcinomas was ~2.5-fold and for TCA induced carcinomas ~2.0-fold. Again the authors state that "if corrected for nonspecific hybridization, carcinomas expressed approximately 4 times as much c-H-ras than observed in surrounding tissues" Given that control and nontumor tissue results were given as the controls for the expression increases observed in "lesions," it is unclear what this the usefulness of this "correction" is. The authors reported that "focal areas of increased expression of c-H-ras were not observed within carcinomas."

The limitations of this experiment include uncertainty as to what doses were used and how many animals were exposed to produce animals with tumors. In addition results of differing doses were pooled and the term hyperplastic nodule, undefined. The authors state that c-Myc expression in itself is not sufficient for transformation and that its over expression commonly occurs in malignancy. They also state that "Unfortunately, the limited amount of tissue available prevented a more serious pursuit of this question in the present study." In regard to the effects of cessation of exposure, the authors do not present data on how many animals were tested with the cessation protocol, what doses were used, and how many lesions comprised their results and thus, comparisons between these results and those from 52 weeks of continuous exposure are hard to make. Quantitatively, the small number of animals, whose lesions were tested, was $n = 2-4$ for the cessation groups. Bull et al. (1990) is given as the source of data for the cessation experiment (see Section E.2.3.2.1, above).

E.2.3.2.6. ***DeAngelo et al., 1999.*** The focus of this study was to "determine a dose response for the hepatocarcinogenicity of DCA in male mice over a lifetime exposure and to examined several modes of action that might underlie the carcinogenic process." As DeAngelo et al pointed out, many studies of DCA had been conducted at high concentrations and for less than lifetime studies, and therefore, of suspect relevance to environmental concentrations. This study is one of the few that examined DCA at a range of exposure concentrations to determine a dose-response in mice. The authors concluded that DCA-induced carcinogenesis was not dependent on peroxisome proliferation or chemically sustained proliferation. The number of hepatocellular carcinomas/animals was reported to be significantly increased over controls at all DCA treatments including 0.05 g/L and a no-observed-effect level (NOEL) not observed. Peroxisome proliferation was reported to be significantly increased at 3.5 g/L DCA only at 26 weeks and did not correlate with tumor response. No significant treatment effects on labeling of hepatocytes (as a measure of proliferation) outside proliferative lesions were also reported and thus, that DCA-induced liver cancer was not dependent on peroxisome proliferation or chemically sustained cell proliferation.

Male B6C3F1 mice were 28−30 days of age at the start of study and weighed 18−21 g (or ~14% range). They were exposed to 0, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA via drinking water as a neutralized solution. The time-weighted mean daily water consumption calculated over the 100-week treatment period was reported to be 147, 153, 158, 151, 147, and 124 (84% of controls) mL/kg/day for 0, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively. The number of animals reported as used for interim sacrifices were 35, 30, 30, 30 and 30 for controls, 0.5, 1.0, 2.0, and 3.5 g/L DCA treated groups respectively (i.e., 10 mice per treatment group at interim sacrifices of 26, 52 and 78 weeks). The number of animals at final sacrifice were reported to be 50, 33, 24, 32, 14 and 8 for controls, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA treated groups respectively. The number of animals with unscheduled deaths before final sacrifice were reported to be 3, 2, 1, 9, 11 and 8 for controls, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA treated groups respectively. The Authors reported that early mortality tended to occur from liver cancer. The number of animals examined for pathology were reported to be 85, 33, 55, 65, 51, and 41 for controls, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA treated groups respectively. The experiment was conducted in two parts with control, 0.5, 1.0 L, 2.0, and 3.5 g/L groups treated and then 1 months later a second group consisting of 30 control group mice and 35 mice in a 0.05 g/L DCA exposure group studied. The authors reported not difference in prevalence and multiplicity of hepatocellular neoplasms in the two groups so that data were summed and reported together. The number of animals reported as examined for tumors were *n* = 10 animals, with controls reported to be 35 animals split among 3 interim sacrifice times—exact number per sacrifice time is unknown. The number of animals reported “with pathology” and assumed to be included in the tumor analyses from Table 1, and the sum of the number of animals “scheduled for sacrifice that survived till 100 weeks” and “interim sacrifices” do not equal each other. For the 1 g/L DCA exposure group, 30 animals were sacrificed at interim periods, 32 animals were sacrificed at 100 weeks, 9 animals were reported to have unscheduled deaths, but of those 71 animals only 65 animals were reported to have pathology for the group. Therefore, some portion of animals with unscheduled deaths must have been included in the tumor analyses. The exact number of animals that may have died prematurely but included in analyses of pathology for the 100 week group is unknown. In Figure 3 of the study, the authors reported prevalence and multiplicity of hepatocellular carcinomas following 79 to 100 weeks of DCA exposure in their drinking water. The number of animals in each dose group used in the tumor analysis for 100 weeks was not given by the authors. Given that the authors included animals that survived past the 78 interim sacrifice period but died unscheduled deaths in their 100 week results, the number must have been greater than those reported as present at final sacrifice. A comparison of the data for the 100-week data presented in Table 3a and Figure 3 shows that the data reported for 100 weeks is actually for animals that survived from 79 to 100 weeks. The authors report a dose-response that is statistically significant from 0.5 to 3.5 g/L DCA for hepatocellular carcinoma incidence and a dose-response in hepatocellular carcinoma multiplicity that is significantly increased over controls from 0.05 to 0.5 g/L DCA that survived 79 to 100 weeks of exposure (i.e., 0, 8-, 84-, 168-, 315-, and 429 mg/kg/d dose groups with prevalences of 26, 33, 48, 71, 95, and 100%, respectively, and multiplicities of 0.28, 0.58, 0.68, 1.29, 2.47, and 2.90, respectively). Hepatocellular adenoma incidence or multiplicity was not reported for the 0.05 g/L DCA exposure group.

In Table 3 of the report, the time course of hepatocellular carcinomas and adenoma development are given and summarized in Table E-2, below.

The authors reported hepatocellular carcinomas and number of lesions/animal in mice that survived 79−100 weeks of exposure (they combined exposure groups to be animals after the Week 78 sacrifice time that did and did not make it to 100 weeks). This is the same data reported above for the 100 week exposure with the inclusion of the 0.05 g/L DCA data. The difference between number of animals at interim and final sacrifices and those “with pathology” and used in the tumor analysis but most likely coming from unscheduled deaths is reported in Table E-3 as “extra” and varied across treatment groups.

Table E-2. Prevalence and Multiplicity data from DeAngelo et al. (1999)

Prevalence	Multiplicity (lesions/animal *m* ± SEM)	
	Carcinomas	**Adenomas**
52 weeks control = 0% carcinomas, 0% adenoma	0	0
0.5 g/L DCA = 0/10 carcinoma, 1/10 adenomas	0	0.10 ± 0.09
1.0 g/L DCA = 0/10 carcinomas, 1/10 adenomas	0	0.10 ± 0.09
2.0 g/L DCA = 2/10 carcinomas, 0/10 adenomas	0.20 ± 0.13	0
3.5 g/L DCA = 5/10 carcinomas, 5/10 adenomas	0.70 ± 0.25	0.80 ±0.31
78 weeks control = 10% carcinomas, 10% adenomas	0.10 ± 0.10	0.10 ± 0.09
0.5 g/L DCA = 0/10carcinoma, 1/10 adenomas	0	0.10 ± 0.09
1.0 g/L DCA = 2/10 carcinomas, 2/10 adenomas	0.20 ± 0.13	0.20 ± 0.13
2.0 g/L DCA = 5/10 carcinomas, 5/10 adenomas	1.0 ± 0.47	1.00 ± -0.42
3.5 g/L DCA = 7/10 carcinomas, 5/10 adenomas	1.20 ± 0.37	1.00 ± 0.42
100 weeks control = 26% carcinoma, 10% adenoma	0.28 ± 0.07	0.12 ± 0.05
0.5 g/L DCA = 48% carcinoma, 20% adenomas	0. 68 ± 0.17	0.32 ± 0.14
1.0 g/L DCA = 71% carcinomas, 51.4% adenomas	1.29 ± 0.17	0.80 ± 0.17
2.0 g/L DCA = 95% carcinomas, 42.9% adenomas	2.47 ± 0.29	0.57 ± 0.16
3.5 g/L DCA = 100% carcinomas, 45% adenomas	2.90 ± 0.40	0.64 ± 0.23

Table E-3. Difference in pathology by inclusion of unscheduled deaths from DeAngelo et al. (1999).

Dose = Prevalence of HC	#HC/animal	*n* = at 100 wk	Extra added in
Control = 26%	0.28	50	0
0.05 g/L = 33%	0.58	33	0
0.5 g/L = 48%	0.68	24	1
1 g/L = 71%	1.29	32	3
2 g/L = 95%	2.47	14	7
3.5 g/L = 100%	2.9	8	3

These data show a dose-related increase in tumor formation and decrease in time-to-tumor associated with DCA exposure at the lowest levels examined. These findings are limited by the small number of animals examined at 100 weeks but especially those examined at “interim sacrifice” periods (n = 10). The data illustrate the importance of examining multiple exposure levels at lower concentrations at longer durations of exposure and with an adequate number of animals to determine the nature of a carcinogenic response.

Preneoplastic and non-neoplastic hepatic changes were reported to have been described previously and summarized as large preneoplastic foci observed at 52 weeks with multiplicities of 0.1, 0.1, 0.2 and 0.16 for 0.5, 1, 2, and 3.5 g/L DCA exposure respectively. At 100 weeks all values were reported to be significant (0.03, 0.06, 0.14, 0.27 for 0.5, 1, 2, and 3.5 g/L DCA exposure respectively). Control values were not reported by the authors. The authors reported that the prevalence and severity of hepatocellular cytomegaly and of cytoplasmic vacuolization with glycogen deposition to be dose-related and considered significant in all dose groups examined when compared to control liver. However, no quantitative data were shown. The authors reported a severity index of 0 = none, 1 = ≤25%, 2 = 50−75% and 4 = 75% of liver section for hepatocellular necrosis and report at 26 weeks scores (n = 10 animals) of 0.10 ± 0.10, 0.20 ± 0.13, 1.20 ± 0.38, 1.20 ± 0.39 and 1.10 ± 0.28 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively. Thus, there appeared to be a treatment but not dose-related increase in hepatocellular necrosis that is does not involve most of the liver from 1 to 3.5 g/L DCA at this time point. At 52 weeks of exposure the score for hepatocellular necrosis was reported to be 0, 0, 0.20 ± 0.13, 0.40 ± 0.22 and 1.10 ± 0.43 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively. At 78 weeks of exposure the score for hepatocellular necrosis was reported to be 0, 0, 0, 0.30 ± 0.21 and 0.20 ± 0.13 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively. Finally, the final sacrifice time when more animals were examined the extent of hepatocellular necrosis was reported to be 0.20 ± 0.16, 0.20 ± 0.08, 0.42 ± 0.15, 0.38 ± 0.20 and 1.38 ± 0.42 for control, 0.5, 1, 2, and 3.5 g/L DCA treatment groups, respectively. Thus, there was not reported increase in hepatocellular necrosis at any exposure period for 0.5 g/L DCA treatment and the mild hepatocellular necrosis seen at the three highest exposure concentrations at 26 weeks had diminished with further treatment except for the highest dose at up to100 weeks of treatment. Clearly the pattern of hepatocellular necrosis did not correlate with the dose-related increases in hepatocellular carcinomas reported by the authors and was not increased over control at the 0.5 g/L DCA level where there was a DCA-related tumor increase.

The authors cite previously published data and state that CN-insensitive palmitoyl CoA oxidase activity (a marker of peroxisome proliferation) data for the 26 week time point plotted against 100 weeks hepatocellular carcinoma prevalence of animals bearing tumors was significantly enhanced at concentrations of DCA that failed to induce “hepatic PCO” activity. The authors report that neither 0.05 nor 0.5 g/L DCA had any marked effect on PCO activity and that it was "only significantly increased after 26 weeks of exposure to 3.5 g/L DCA and returned to control level at 52 weeks (data not shown)." In regards to hepatocyte labeling index after treatment for 5 days with tritiated thymidine, the authors report that animals examined in the dose-response segment of the experiment at 26 and 52 weeks were examined but no details of the analysis were reported. The authors comment on the results from this study and a previous one that included earlier time points of study and stated that there were "no significant alterations in the labeling indexes for hepatocytes outside of proliferative lesions at any of the DCA concentrations when compared to the control values with the exception of 0.05 g/L DCA at 4 weeks (4.8 ± 0.6 vs. 2.7 ± 0.4 control value; data not shown)."

The effects of DCA on body weight, absolute liver weight and percent liver/body weight were given in Table 2 of the paper for 26, 52, 78 and 100 weeks exposure. For 52 and 78 week studies 10 animals per treatment group were examined. Liver weights were not determined for the lowest exposure concentration (0.05 g/L DCA) except for the 100 week exposure period. At 26 weeks of exposure there was not a statistically significant change in body weight among the exposure groups (i.e., 35.4 ± 0.7, 37.0 ± 0.8, 36.8 ± 0.8, 37.9 ± 0.6, and 34.6 ± 0.8 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight was reported to have a dose-related significant increase in comparison to controls at all exposure concentrations examined with liver weight reaching a plateau at the 2 g/L concentration (i.e., 1.86 ± 0.07, 2.27 ± 0.10, 2.74 ± 0.08, 3.53 ± 0.07, and 3.55 ± 0.1 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase (i.e., 5.25% ± 0.11%, 6.12% ± 0.16%, 7.44% ± 0.12%, 9.29% ± 0.08%, and 10.24% ± 0.12% for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). This represented a 1.17-, 1.41-, 1.77-, and 1.95-fold of control percent liver/body weight at these exposures at 26 weeks.

At 52 weeks of exposure there was not a statistically significant change in body weight among the exposure groups except for the 3.5 g/L exposed group in which there was a significant decrease in body weight (i.e., 39.9 ± 0.8, 41.7 ± 0.8, 41.7 ± 0.9, 40.8 ± 1.0, and 35.0 ± 1.1 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight was reported to have a dose-related significant increase in comparison to controls at all exposure concentrations examined with liver weight reaching a plateau at the 2 g/L concentration (i.e., 1.87 ± 0.13, 2.39 ± 0.04, 2.92 ± 0.12, 3.47 ± 0.13, and 3.25 ± 0.24 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase (i.e., 4.68% ± 0.30%, 5.76% ± 0.12%, 7.00% ± 0.15%, 8.50% ± 0.26%, and 9.28% ± 0.64% for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). For liver weight and percent liver/body weight there was much larger variability between animals within the treatment groups compared to controls and other treatment groups. There were no differences reported for patterns of change in body weight, absolute liver weight, and percent liver/body weight between animals examined at 26 weeks and those examined at 52 weeks. At 78 weeks of exposure there was not a statistically significant change in body weight among the exposure groups except for the 3.5 g/L exposed group in which there was a significant decrease in body weight (i.e., 46.7 ± 1.2, 43.8 ± 1.5, 43.4 ± 0.9, 42.3 ± 0.8, and 40.2 ± 2.2 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight was reported to have a dose-related increase in comparison to controls at all exposure concentrations examined but none were reported to be statistically significant (i.e., 2.55 ± 0.14, 2.16 ± 0.09, 2.54 ± 0.36, 3.31 ± 0.63, and 3.93 ± 0.59 g for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase over control values but only the 3.5 g/L exposure level was reported to be statistically significant (i.e., 5.50% ± 0.35%, 4.93% ± 0.09%, 5.93% ± 0.97%, 7.90% ± 1.55%, and 10.14% ± 1.73% for control, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Finally, for the animals reported to be sacrificed between 90 and 100 weeks there was not a statistically significant change in body weight among the exposure groups except for the 2.0 and 3.5 g/L exposed groups in which there was a significant decrease in body weight (i.e., 43.9 ± 0.8, 43.3 ± 0.9, 42.1 ± 0.9, 43.6 ± 0.7, 36.1 ± 1.2, and 36.0 ± 1.3 g for control, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively). Absolute liver weight did not show a dose-response pattern at the two lowest exposure levels but was elevated with the 3 highest doses with the two highest being statistically significant (i.e., 2.59 ± 0.26, 2.74 ± 0.20, 2.51 ± 0.24, 3.29 ± 0.21, 4.75 ± 0.59, and 5.52 ± 0.68 g for control, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively). The percent liver/body weight ratio increases due to DCA exposure were reported to have a similar pattern of increase over control values but only the 2.0 and 3.5 g/L exposure levels were reported to be statistically significant (i.e., 6.03% ± 0.73%, 6.52% ± 0.55%, 6.07% ± 0.66%, 7.65% ± 0.55%, 13.30% ± 1.62%, and 15.70% ± 2.16% for control, 0.05, 0.5, 1, 2, and 3.5 g/L DCA, respectively).

It must be recognized that liver weight increases, especially in older mice, will reflect increased weight due to tumor burden and thus, DCA-induced hepatomegaly will be somewhat obscured at the longer treatment durations. However, by 100 weeks of exposure there did not appear to be an increase in liver weight at the 0.05 and 0.5 g/L exposures while there was an increase in tumor burden reported. Examination of the 0.5 g/L exposure group from 26 to 100 weeks shows that slight hepatomegaly, reported as either absolute liver weight increase over control or change in percent liver/body ratio, was present by 26 weeks (i.e., 22% increase in liver weight and 17% increase in percent liver/body weight), decreased with time, and while similar at

52 weeks, was not significantly different from control values at 78 or 100 weeks durations of exposure. However, tumor burden was increased at this low concentration of DCA.

The authors present a figure comparing the number of hepatocellular carcinomas per animal at 100 weeks compared with the percent liver/body weight at 26 weeks and show a linear correlation (r^2 = 0.9977). Peroxisome proliferation and DNA synthesis, as measured by tritiated thymidine, were reported to not correlate with tumor induction profiles and were also not correlated with early liver weight changes induced by DCA exposure. Most importantly, in a paradigm that examined tumor formation after up to 100 weeks of exposure, DCA-induced tumor formation was reported to occur at concentrations that did not also cause cytotoxicity and at levels 20 to 40 times lower than those used in "less than lifetime" studies reporting concurrent cytotoxicity.

E.2.3.2.7. ***Carter et al., 2003.*** The focus of this study was to present histopathological analyses that included classification, quantification and statistical analyses of hepatic lesions in male B6C3F1 mice receiving DCA at doses as low as 0.05 g/L for 100 weeks and at 0.5, 1.0, 2.0, and 3.5 g/L for between 26 and 100 weeks. This analysis used tissues from the DeAngelo et al. (1999) (two blocks from each lobe and all lesions found at autopsy). This study used the following diagnostic criteria for hepatocellular changes. Altered hepatic Foci (AHF) were defined as histologically identifiable clones that were groups of cells smaller than a liver lobule that did not compress the adjacent liver. Large foci of cellular alteration (LFCA) were defined as lesions larger than the liver lobule that did not compress the adjacent architecture (previously referred to as hyperplastic nodules by Bull et al., 1990) but had different staining. These are not non-neoplastic proliferative lesions termed "hepatocellular hyperplasia" that occur secondary to hepatic degeneration or necrosis. Adenomas (ADs) showed growth by expansion resulting in displacement of portal triad and had alterations in both liver architecture and staining characteristics. Carcinomas (CAs) were composed of cells with a high nuclear-to-cytoplasmic ration and with nuclear pleomorphism and atypia that showed evidence of invasion into the adjacent tissue. They frequently showed a trabecular pattern characteristic of mouse hepatocellular CAs.

The report grouped lesions as eosinophilic, basophilic and/or clear cell, and dysplastic. "Eosinophilic lesions included lesions that were eosinophilic but could also have clear cell, spindle cell or hyaline cells. Basophilic lesions were grouped with clear cell and mixed cell (i.e., mixed basophilic, eosinophilic, hyaline, and/or clear cell) lesions." The authors reported that

> this grouping was necessary because many lesions had both a basophilic and clear cell component and a few <10 % had an eosinophilic or hyaline component…Lesions with foci of cells displaying nuclear pleomorphism, hyperchromasia, prominent nucleoli, irregular nuclear borders and/or altered nuclear to cytoplasmic ratios were considered dysplastic irrespective of their tinctorial characteristics.

Therefore, Carter et al. (2003) lumped mixed phenotype lesions into the basophilic grouping so that comparisons with the results of Bull et al. (2002) or Pereira (1996), which segregate mixed phenotype from those without mixed phenotype, cannot be done.

This report examined type and phenotype of preneoplastic and neoplastic lesions pooled across all time points. Therefore, conclusions regarding what lesions were evolving into other lesions have left out the factor of time. Bannasch (1996) reported that examining the evolution of foci through time is critical for discerning neoplastic progression and described foci evolution from eosinophilic or basophilic lesions to more basophilic lesions. Carter et al. (2003) suggest that size and evolution into a more malignant state are associated with increasing basophilia, a conclusion consistent with those of Bannasch (1996). The analysis presented by Carter et al. (2003) also suggested that there was more involvement of lesions in the portal triad, which may give an indication where the lesions arose. Consistent with the results of DeAngelo et al. (1999), Carter et al. (2003) reported that "DCA (0.05 – 3.5 g/L) increased the number of lesions per animal relative to animals receiving distilled water and shortened the time to development of all classes of hepatic lesions." They also concluded that

> although this analysis could not distinguish between spontaneously arising lesions and additional lesions of the same type induced by DCA, only lesions of the kind that were found spontaneously in control liver were found in increased numbers in animals receiving DCA…Development of eosinophilic, basophilic and/or clear cell and dysplastic AHF was significantly related to DCA dose at 100 weeks and overall adjusted for time.

The authors concluded that the presence of isolated, highly dysplastic hepatocytes in male B6C3F1 mice chronically exposed to DCA suggested another direct neoplastic conversion pathway other than through eosinophilic or basophilic foci.

It appears that the lesions being characterized as carcinomas and adenomas in DeAngelo et al. (1999) were not the same as those by Carter et al. (2003) at 100 weeks even though they were from the same tissues (see Table E-4). Carter et al. identified all carcinomas as dysplastic despite tincture of lesion and subdivided adenomas by tincture. If the differing adenoma multiplicities are summed for Carter et al. they do not add up to the same total multiplicity of adenoma given by DeAngelo et al. It is unclear how many animals were included in the differing groups in both studies for pathology. The control and high-dose groups differ in respect to “animals with pathology” between DeAngelo et al. and the “number of animals in groups” examined for lesions in Carter et al. Neither report gave how many animals with unscheduled deaths were treated in regards to how the pathology data were included in presentation of results. Given that DeAngelo et al. represents animals at 100 weeks as also animals from 79−100 weeks exposure, it is probable that the animals that died after 79 weeks were included in the group of animals sacrificed at 100 weeks. However, the number of animals affecting that result (which would be a mix of exposure times) for either DeAngelo et al., or Carter et al., is unknown from published reports. In general, it appears that Carter et al. (2003) reported more adenomas/animal for their 100 week animals than DeAngelo et al. (1999) did, while DeAngelo et al. reported more carcinomas/animal. Carter et al. reported more adenomas/animal than controls while DeAngelo et al. reported more carcinomas/animal than controls at 100 weeks of exposure.

Table E-4. Comparison of data from Carter et al. (2003) and DeAngelo et al. (1999)

Exposure level of DCA at 79−100 wk (g/L)	Total adenoma multiplicity (Carter)	Total adenoma multiplicity (DeAngelo)	Total carcinoma multiplicity (Carter)	Total carcinoma multiplicity (De Angelo)	Sum of adenomas and carcinoma multiplicity (Carter)	Sum of adenomas and carcinoma multiplicity (DeAngelo)
0	0.22	0.12	0.05	0.28	0.27	0.40
0.05	0.48	-	<0.025	0.58	~0.50	-
0.5	0.44	0.32	0.20	0.68	0.64	1.0
1.0	0.52	0.80	0.30	1.29	0.82	2.09
2.0	0.60	0.57	1.55	2.47	2.15	3.27
3.5	1.48	0.64	1.30	2.90	2.78	3.54

In order to compare these data with others (e.g., Pereira, 1996) for estimates of multiplicity by phenotype or tincture it would be necessary to add foci and LFCA together as foci, and adenomas and carcinomas together as tumors. It would also be necessary to lump mixed foci together as “basophilic” from other data sets as was done for Carter et al. in describing “basophilic lesions.” If multiplicity of carcinomas and adenomas are summed from each study to control for differences in identification between adenoma and carcinoma, there are still differences in the two studies in multiplicity of combined lesions/animal with DeAngelo giving consistently higher estimates. However, both studies show a dose response of tumor multiplicity with DCA and a difference between control values and the 0.05 DCA exposure level. Error is introduced by having to transform the data presented as a graph in Carter et al. (2003). Also no SEM is given for the Carter data.

In regard to other histopathological changes, the authors report that

> necrosis was found in 11.3% of animals in the study and the least prevalent toxic or adaptive response. No focal necrosis was found at 0.5 g/L. The incidence of focal necrosis did not differ from controls at 52 or 78 weeks and only was greater than controls at the highest dose of 3.5 g/L at 100 weeks. Overall necrosis was negatively related to the length of exposure and positively related to the DCA dose. Necrosis was an early and transitory response. There was no difference in necrosis 0 and 0.05 g/L or 0.5 g/L. There was an increase in glycogen at 0.5 g/L at the perioportal area. There was no increase in steatosis but a dose-related decrease in steatosis. Dysplastic LFCA were not related to necrosis indicating that these lesions do not represent, regenerative or reparative hyperplasia. Nuclear atypia and glycogen accumulation were associated with dysplastic adenomas. Necrosis was not related to occurrence of dysplastic adenomas. Necrosis was of borderline significance in relation to presence of hepatocellular carcinomas. Necrosis was not associated with dysplastic LFCAs or Adenomas.

They concluded that "the degree to which hepatocellular necrosis underlies the carcinogenic response is not fully understood but could be significant at higher DCA concentrations ($\geq$1g/L)."

E.2.3.2.8. ***Stauber and Bull, 1997.*** This study was designed to examine the differences in phenotype between altered hepatic foci and tumors induced by DCA and TCA. Male B6C3F1 mice (7 weeks old at the start of treatment) were treated with 2.0 g/L neutralized DCA or TCA in drinking water for 38 or 50 weeks, respectively. They were then treated with additional exposures (n = 12) of 0, 0.02, 0.1, 0.5, 1.0, 2.0 g/L DCA or TCA for an additional 2 weeks. Three days prior to sacrifice in DCA-treated mice or 5 days for TCA-treated mice, animals had miniosmotic pumps implanted and administered BrdU. Immunohistochemical staining of hepatocytes from randomly selected fields (minimum of 2,000 nuclei counter per animal) from 5 animals per group were reported for 14- and 28-day treatments. It was unclear how many animals were examined for 280- and 350-day treatments from the reports. The percentage of labeled cells in control livers was reported to vary between 0.1 and 0.4% (i.e., 4-fold). There was a reported ~3.5-fold of control level for TCA labeling at 14 day time period and a ~5.5-fold for DCA. At 28 days there was ~2.5-fold of control for TCA but a ~2.3-fold decrease of control for DCA. At 280 days there was no data reported for TCA but for DCA there was a ~2-fold decrease in labeling over control. At 350 days there was no data for DCA but a reported ~2.3-fold decrease in labeling of control with TCA. The authors reported that the increases at Day 14 for TCA and DCA exposure and the decrease at Day 28 for DCA exposure were statistically significant although a small number of animals were examined. Thus, although there may be some uncertainty in the exact magnitude of change, there was at most ~5-fold of control labeling for DCA within after 14 days of exposure that was followed by a decrease in DNA synthesis by Day 28 of treatment. These data show that hepatocytes undergoing DNA synthesis represented a small population of hepatocytes with the highest level with either treatment less than 1% of hepatocytes. Rates of cell division were reported to be less than control for both DCA and TCA by 40 and 52 weeks of treatment.

In this study the authors reported that there was no necrosis with the 2.0 g/L DCA dose for 52 weeks and conclude that necrosis is a recurring but inconsistent result with chronic DCA treatment. Histological examination of the livers involved in the present study found little or no evidence of such damage or overt cytotoxicity. It was assumed that this effect has little bearing on data on replication rates. Foci and tumors were combined in reported results and therefore, cannot be compared the results Bull et al. (2002) or to DeAngelo et al. (1999). Prevalence rates were not reported. Data were reported in terms of "lesions" with DCA-induced "lesions" containing a number of smaller lesions that were heterogeneous and more eosinophilic with larger "lesions" tending to less numerous and more basophilic. For TCA results using this paradigm, the "lesions" were reported to be less numerous, more basophilic, and larger than those induced by DCA. The DCA-induced larger "lesions" were reported to be more "uniformly reactive to c-Jun and c-Fos but many nuclei within the lesions displaying little reactivity to c-Jun." The authors stated that while most DCA-induced "lesions" were homogeneously immunoreactive to c-Jen and C-Fos (28/41 lesions), the rest were stained heterogeneously. For TCA-induced lesions, the authors reported not difference in staining between "lesions" and normal hepatocytes in TCA-treated animals. Again, of note is that not only were "lesions" comprised of foci and tumors at different stages of progression reported in these results, but that also DCA and TCA results were reported for different durations of exposure.

E.2.3.2.9. ***Pereira, 1996.*** The focus of this study was to report the dose-response relationship for the carcinogenic activity of DCA and TCA in female B6C3F1 mice and the characteristics of the lesions. Female B6C3F1 mice (7−8 weeks of age) were given drinking water with either DCA or TCA at 2.0, 6.67, or 20 mmol/L and neutralized with sodium hydroxide to a pH or 6.5−7.5. The control received 20 mmol/L sodium chloride. Conversion of mmol/L to g/L was as follows: 20.0 mmol/L DCA = 2.58 g/L, 6.67 mmol/L DCA = 0.86 g/L, 2.0 mmol/L = 0.26 g/L, 20.0 mmol/L TCA = 3.27 g/L, 6.67 mmol/L TCA = 1.10 g/L, 2.0 mmol/L TCA = 0.33 g/L. The concentrations were reported to be chosen so that the high concentration was comparable to those previously used by us to demonstrate carcinogenic activity. The mice were exposed till sacrifice at 360 (51 weeks), or 576 days (82 weeks) of exposure. Whole liver was reported to be cut into ~3 mm blocks and along with representative section of the visible lesions fixed and embedded in paraffin and stained with H&E for histopathological evaluation of foci of altered hepatocytes, hepatocellular adenomas, and hepatocellular carcinomas. The slides were reported to be evaluated blind. Foci of altered hepatocytes in this study were defined as containing 6 or more cells and hepatocellular adenomas were distinguished from foci by the occurrence of compression at greater than 80% of the border of the lesion.

Body weights were reported to be decreased only the highest dose of DCA from 40 weeks of treatment onward. For TCA there were only 2 examination periods (Weeks 51 and 82) that had significantly different body weights from control and only at the highest dose. Liver/body weight percentage was reported in comparison to concentration graphically and shows a dose-response for DCA with steeper slope than that of TCA at 360 and 576 days of exposure. The authors report that all three concentrations of DCA resulted in increased vacuolation of hepatocytes.(probably due to glycogen removal from tissue processing). Using a score of 1−3, (with 0 indicating the absence of vacuolization, +1 indicating vacuolated hepatocytes in the periportal zone, + 2 indicating distribution of vacuolated hepatocytes in the midzone, and +3 indicating maximum vacuolization of hepatocytes throughout the liver), the authors also reported "the extent of vacuolization of the hepatocytes in the mice administered 0, 2.0, 6.67 or 20.0 mmol/l DCA was scored as 0.0, 0.80 ± 0.08, 2.32 ± 0.11, or 2.95 ± 0.05, respectively."

Cell proliferation was reported to be determined in treatment groups containing 10 mice each and exposed to either DCA or TCA for 5, 12, or 33 days with animals implanted with miniosmotic pumps 5 days prior to sacrifice and administered BrdU. Tissues were immunohistochemically stained for BrdU incorporation. At least 2,000 hepatocytes/mouse were reported to be evaluated for BrdU-labeled and unlabeled nuclei and the BrDU-labeling index was calculated as the percentage of hepatocytes with labeled nuclei. Pereira (1996) reported a dose-related increase in BrDU labeling in 2,000 hepatocytes that was statistically significant at 6.67 and 20.mmol/L DCA at 5 days of treatment but that labeling at all exposure concentrations decreased to control levels by Day 12 and 33 of treatment. The largest increase in BrdU labeling was reported to be a 2-fold of controls at the highest concentration of DCA after 5 days of exposure. For TCA all doses (2.0, 6.67 and 20 mmol/L) gave a similar and statistically significant increase in BrDU labeling by 5 days of treatment (~3-fold of controls) but by days 12 and 33 there were no increases above control values at any exposure level. Given the low level of hepatocyte DNA synthesis in quiescent control liver, these results indicate a small number of hepatocytes underwent increased DNA synthesis after DCA or TCA treatment and that by 12 days of treatment these levels were similar to control levels in female B6C3F1 mice.

Incidence of foci and tumors in mice administered DCA or TCA (prevalence or number of animals with tumors of those examined at sacrifice) in this report are given below in Tables E-5 and E-6.

Table E-5. Prevalence of foci and tumors in mice administered NaCl, DCA, or TCA from Pereira (1996)

Treatment	*N*	Foci		Adenomas		Carcinomas	
		Number	%	Number	%	Number	%
82 wks							
20.0 mmol NaCl	90	10	11.1	2	2.2	2	2.2
20.0 mmol DCA	19	17	89.5*	16	84.2*	5	26.3*
6.67 mmol DCA	28	11	39.3*	7	25.0*	1	3.6
2.0 mmol DCA	50	7	14.0	3	6.0	0	0
20.0 mmol TCA	18	11	61.1*	7	38.9*	5	27.8%*
6.67 mmol TCA	27	9	33.3*	3	11.1	5	18.5*
2.0 mmol TCA	53	10	18.9	4	7.6	0	0
51 wks							
20.0 mmol NaCl	40	0	0	1	2.5	0	0
20.0 mmol DCA	20	8	40.0*	7	35*	1	5
6.67 mmol DCA	20	1	5	3	15	0	0
2.0 mmol DCA	40	0	0	0	0	0	0
20.0 mmol TCA	20	0	0	2	15.8	5	25*
6.67 mmol TCA	19	0	0	3	7.5	0	0
2.0 mmol TCA	40	3	7.5	3	2.5	0	0

*$p < 0.05$.

NaCl = sodium chloride control.

Table E-6. Multiplicity of foci and tumors in mice administered NaCl, DCA, or TCA from Pereira (1996)

Treatment	*N*	Foci/mouse	Adenomas/mouse	Carcinomas/mouse
82 wks				
20.0 mmol NACL	90	0.11 ± 0.03	0.02 ± 0.02	0.02 ± 0.02
20.0 mmol DCA	19	7.95 ± 2.00[a]	5.58 ± 1.14[a]	0.37 ± 0.17[b]
6.67 mmol DCA	28	0.39 ± 0.11[b]	0.32 ± 0.13[b]	0.04 ± 0.04
2.0 mmol DCA	50	0.14 ± 0.05	0.06 ± 0.03	0
20.0 mmol TCA	18	1.33 ± 0.31[a]	0.61 ± 0.22[b]	0.39 ± 0.16[b]
6.67 mmol TCA	27	0.41 ± 0.13[b]	0.11 ± 0.06	0.22 ± 0.10[b]
2.0 mmol TCA	53	0.26 ± 0.08	0.08 ± 0.04	0
51 wks				
20.0 mmol NACL	40	0	0.03 ± 0.03	0
20.0 mmol DCA	20	0.60 ± 0.22[a]	0.45 ± 0.17[a]	0.10 ± 0.10
6.67 mmol DCA	20	0.05 ± 0.05	0.20 ± 0.12	0
2.0 mmol DCA	40	0	0	0
20.0 mmol TCA	20	0	0.15 ± 0.11	0.50 ± 0.18[b]
6.67 mmol TCA	19	0	0.21 ± 0.12	0
2.0 mmol TCA	40	0.08 ± 0.04	0.08 ± 0.04	0

[a]$p < 0.01$.
[b]$p < 0.05$.

NaCl = sodium chloride control.

These data show the decreased power of using fewer than 50 mice, especially at shorter durations of exposure. By 82 weeks of exposure increased adenoma and carcinomas induced by TCA or DCA treatment are readily apparent.

The foci of altered hepatocytes and the tumors obtained from this study were reported to be basophilic, eosinophilic, or mixed containing both characteristics and are shown in Tables E-7 and E-8. DCA was reported to induce a predominance of eosinophilic foci and tumors, with over 80% of the foci and 90% of the tumors in the 6.67 and 20.0 mmol/L concentration groups being eosinophilic. Only approximately half of the lesions were characterized as eosinophilic with the rest being basophilic in the group administered 2.0 mmol/L DCA. The eosinophilic foci and tumors were reported to consistently stained immunohistochemically for the presence of GST-π, while basophilic lesions did not stain for GST-π, except for a few scattered cells or small areas comprising less than 10% of foci. The foci of altered hepatocytes in the TCA treatment groups were approximately equally distributed between basophilic and eosinophilic in tincture. However, the tumors were predominantly basophilic lacking GST-pi (21 of 28 or 75%) including all 11 hepatocellular carcinomas. The limited numbers of lesions, i.e., 14, in the sodium chloride (vehicle control) group were characterized as 64.3, 28.6, and 7.1% basophilic, eosinophilic, and mixed, respectively.

Table E-7. Phenotype of foci reported in mice exposed to NaCl, DCA, or TCA by Pereira (1996)

Treatment at 51 and 82 wk	*N*	% Foci		
		Basophilic	Eosinophilic	Mixed
20.0 mmol NaCl	10	70	30	0
20.0 mmol DCA	150	3.3	96.7	0
6.67 DCA	11	18.2	81.8	0
2.0 mmol DCA	7	42.8	57.2	0
20.0 mmol TCA	22	36.4	54.6	9.1
6.67 mmol TCA	11	45.5	54.5	0
2.0 mmol TCA	13	38.5	61.5	0

NaCl = sodium chloride control.

Table E-8. Phenotype of tumors reported in mice exposed NaCl, DCA, or TCA by Pereira (1996)

Treatment at 51 and 82 wk	*N*	Tumors		
		Basophilic	Eosinophilic	Mixed
20.0 mmol NaCl	4	50	25	25.5
20.0 mmol DCA	105	2.9	96.1	1
6.67 DCA	10	10	90	0
2.0 mmol DCA	3	0	100	0
20.0 mmol TCA	18	61.1	22.2	16.7
6.67 mmol TCA	6	100	0	0
2.0 mmol TCA	4	100	0	0

NaCl = sodium chloride control.

These data for female B6C3F1 mice shows that DCA and TCA treatment induced a mixture of basophilic or eosinophilic foci. The pooling of the data between time and adenoma versus carcinoma decreases the ability to ascertain the phenotype of tumor due to treatment or the progression of phenotype with time as well as the small number of tumor examined at lower exposure concentrations. Foci that occurred at 51 and 82 weeks were presented as one result. Adenomas and carcinoma data were pooled as one endpoint (*n* = number of total foci or tumors examined). Therefore, evolution of phenotype between less to more malignant stages of tumor were lost.

E.2.3.2.10. ***Pereira and Phelps, 1996.*** The focus of this study was to determine tumor response and phenotype in methyl nitrosourea (MNU)-treated mice after DCA or TCA exposure. The concentrations of DCA or TCA were the same as Pereira (1996). For Pereira (1996) the animals were reported to be 7−8 weeks of age when started on treatment and sacrificed after 360 or 576 days of exposure (51 or 82 weeks). For this study and Tao et al. (2004), animals were reported o be 6 weeks of age when exposed to DCA or TCA via drinking water and to be 31 or 52 weeks of age at sacrifice. Thus, exposure time would be ~24 or 45 weeks. A control group of non-MNU treated animals was presented for female B6C3F1 mice treated for 31 or 52 weeks and are discussed in Table E-9, below. Although this paradigm appears to be the same paradigm as those reported in Pereira (1996), fewer animals were studied. The number of animals in each group varied between 8 controls and 14 animals in the 2.0 mmol/L treatment groups. In mice that were not treated with MNU, but were treated with either DCA or TCA at 31 weeks, there were no reported statistically significant treatment-related effect upon the yield of foci or altered hepatocytes and liver tumors but the number of animals examined was small and therefore, of limited power to detect a response. The results below indicate a DCA-related increase in foci and percentage of mice with foci.

See Section E.4.2.3 for further discussion of the results of coexposures to MNU and DCA or TCA from this study.

Table E-9. Multiplicity and incidence data (31 week treatment) from Pereira and Phelps (1996)

Treatment	No	Foci/mouse	incidence %	Adenomas/mouse	incidence %
20.0 mmol NaCl	15	0.13 ± 0.13	6.7	0.13 ± 0.13	not reported
20.0 mmol DCA	10	0.40 ± 0.16	40	0	0
6.67 DCA	10	0.10 ± 0.10	10	0	0
2.0 mmol DCA	15	0	0	0	0
20.0 mmol TCA	10	0	0	0	0
6.67 mmol TCA	10	0	0	0	0
2.0 mmol TCA	15	0	0	0	0

NaCl = sodium chloride control.

E.2.3.2.11. ***Ferreira-Gonzalez et al., 1995.*** The focus of this study was the investigation of differences in H-ras mutation spectra in hepatocellular carcinomas induced by TCA or DCA in male B6C3F1 mice. 28-day old mice were exposed for 104 weeks to 0. 1.0 g or 3.5 g/L DCA or 4.5 g/L TCA that was pH adjusted. Tumors observed from this treatment were diagnosed as either hepatocellular adenomas or carcinomas. DNA was extracted from either spontaneous, DCA- or TCA-induced hepatocellular carcinomas. Samples for analysis were chosen randomly in the treatment groups of which 19% of untreated mice had spontaneous liver hepatocellular carcinomas (0.26 carcinomas/animal), DCA treatment induced 100% prevalence at 3.5 g/L (5.06 carcinomas/animal) and 70.6% carcinomas at 1.0 g/L (1.29 carcinomas/animal). TCA treatment was reported to induce 73.3% prevalence at 4.5 g/L (1.5 carcinomas/animal). The number of samples analyzed was 32 for spontaneous carcinomas, 33 for mice treated with 3.5 g/L DCA, 13 from mice treated with 1.0 g/DCA, and 11 from mice treated with 4.5 g/L TCA. This study has the advantage of comparison of tumor phenotype at the same stage of progression (hepatocellular carcinoma), for allowance of the full expression of a tumor response (i.e., 104 weeks), and an adequate number of spontaneous control lesions for comparison with DCA or TCA treatments. However, tumor phenotype at an endstage of tumor progression reflects of tumor progression and not earlier stages of the disease process.

There were no ras mutations detected except at H-61 in DNA from spontaneously arising tumors of control mice. Only 4/57 samples from carcinogen-treated mice were reported to demonstrate mutation other than in the second exon of H-ras. In spontaneous liver carcinomas, 58% were reported to show mutations in H-61 as compared with 50% of tumor from 3.5 g/L DCA-treated mice and 45% of tumors from 4.5.g/L TCA-treated mice. Thus, there was a heterogeneous response for this phenotypic marker for the spontaneous, DCA-, and TCA-treatment induced hepatocellular carcinomas.

All samples positive for mutation in the exon 2 of H-ras were sequenced for the identification of the base change responsible for the mutation. The authors noted that H-ras mutations occurring in spontaneously developing hepatocellular carcinomas from B6C3F1 male mice are largely confined to codon 61 and involve a change from CAA to either AAA or CGA or CTA in a ratio of 4:2:1. They noted that in this study, all of the H-ras second codon mutations involved a single base substitution in H-61 changing the wild-type sequence from CAA to AAA (80%), CGA (20%) or CTA for the 18 hepatocellular carcinomas examined. In the 16 hepatocellular carcinomas from 3.5 g/L DCA treatment with mutations, 21% were AAA transversions, 50% were CGA transversions, and 29% were CTA transversions. For the 6 hepatocellular carcinomas from 1.0 g/L DCA with mutations, 16% were an AAA transversion, 50% were a CGA transversion, and 34% were a CTA transversion. For the 5 hepatocellular carcinomas from 4.5 g/L TCA with mutations, 80% were AAA transversions, 20% CGA tranversions, and 0% were CTA transversions. The authors note that the differences in frequency between DCA and TCA base substitutions did not achieve statistical significance due to the relatively small number of tumors from TCA-treated mice. They note that the finding of essentially equal incidence of H-ras mutations in spontaneous tumors and in tumors of carcinogen-treated mice did not help in determining whether DCA and TCA acted as "genotoxic" or "nongenotoxic" compounds.

E.2.3.2.12. ***Pereira et al., 2004.*** Pereira et al. (2004) exposed 7−8 week old female B6C3F1 mice treated with "AIN-76A diet" to neutralized 0, or 3.2 g/L DCA in the drinking water and 4.0 or 8.0 g/kg L-methionine added to their diet. The final concentration of methionine in the diet was estimated to be 11.3 and 15.3 g/kg. Mice were sacrifice 8 and 44 weeks after exposure to DCA with body and liver weights evaluated for foci, adenomas, and hepatocellular carcinomas. No histological descriptions were given by the authors other than tinctoral phenotype of foci and adenomas for a subset of the data. The number of mice examined was 36 for the DCA + 8.0 g/kg methionine or 4.0 g/kg methionine group sacrificed at 44 weeks. However, for the DCA-only treatment group the number of animals examined was 32 at 44 weeks and for those groups that did not receive DCA but either methionine at 8.0 or 4.0 g/kg, there were only 16 animals examined. All groups examined at 8 weeks had 8 animals per group. Liver glycogen was reported to be isolated from 30−50 mg of whole liver. Peroxisomal acyl-CoA oxidase activity was reported to be determined using lauroyl-CoA as the substrate and was considered a marker of peroxisomal proliferation. Whole liver DNA methylation status was analyzed using a 5-MeC antibody.

Methionine (8.0 g/kg) and DCA coexposure was reported to result in the death of 3 mice while treatment with methionine (4.0 g/kg) and DCA or methionine (8.0 g/kg) alone was reported to kill one mouse in each group. The authors reported that "There was an increased in body weight during weeks 12 to 36 in the mice that received 8.0 g/kg methionine without DCA. There was no other treatment-related alteration in body weight." However, the authors do not present the data and initial or final body weights were not presented for the differing treatment groups. DCA treatment was reported to increase percent liver/body weight ratios at 8 and 44 weeks to about the same extent (i.e., ~2.4-fold of control at 8 weeks and 2.2-fold of control at 44 weeks). Methionine coexposure was reported to not affect that increase (~2.4-, 2.2-, and 2.1-fold of control after DCA treatment alone, DCA/4 g/kg methionine, and DCA/8 mg/kg methionine treatment for 8 weeks, respectively). There was a slight increase in percent liver/body weight ratio associated with 8.0 g/kg methionine treatment alone in comparison to controls (~7%) at 8 weeks with no difference between the two groups at 44 weeks.

After 8 weeks of only DCA exposure, the amount of glycogen in the liver was reported to be ~2.09-fold of the value for untreated mice (115 vs. 52.5 mg/g glycogen in treated vs. control, respectively at 8 weeks). Both 4 g/kg and 8 g/kg methionine coexposure reduced the amount of DCA-induced glycogen increase in the liver (~1.64-fold of control for DCA/4.0 g/kg methionine and ~1.54-fold of control for DCA/8.0 mg/kg methionine). Thus, for treatment with DCA alone or with the two coexposure levels of methionine, the magnitude of the increase in liver weight was greater than that of the increase in liver glycogen (i.e., 2.42- vs. 2.09-fold of control percent liver/body weight vs. glycogen content for DCA alone, 2.20- vs. 1.64-fold of control percent liver/body weight vs. glycogen content for DCA/4.0 g/kg methionine, 2.10- vs. 1.54-fold of control percent liver/body weight vs. glycogen content for DCA/8.0 g/kg methionine). Thus, the magnitudes of treatment-related increases were higher for percent liver/body weight than for glycogen content in these groups. In regard to percentage of liver mass that glycogen represented, the control value for this study is similar to that presented by Kato-Weinstein et al. (2001) in male mice (~60 mg glycogen per gram liver) and represents ~6% of liver mass. Therefore, a doubling of the amount of glycogen is much less than the 2-fold increases in liver weight observed for DCA exposure in this paradigm. These data suggest that DCA-related increases in liver weight gain are not only the result of increased glycogen accumulation, and that methionine coexposure is affecting glycogen accumulation to a much greater extent than the other underlying processes that are contributing to DCA-induced hepatomegaly after 8 weeks of exposure. The authors reported that 8-weeks of DCA exposure alone did not result in a significant increase in cell proliferation as measured by PCN index (neither data nor methods were shown). This is consistent with other data showing that DCA effects on DNA synthesis were transient and had subsided by 8 weeks of exposure.

The levels of lauroyl-CoA oxidase activity were reported to be increased (~1.33-fold of control) by DCA treatment alone at 8 weeks and to be slightly reduced by 8 g/kg methionine treatment alone (~0.83-fold of control). Methionine coexposure was reported to have little effect on DCA-induced increases in lauroyl-CoA oxidase activity. The levels of DNA methylation were reported to be increased by 8.0 g/kg methionine only treatment at 8 weeks ~1.32-fold of control, and reduced by DCA only treatment to ~0.44-fold of control. DCA and 4.0 g/kg methionine coexposure gave similar results as controls (within 2%). Coexposures of DCA and 8.0 g/kg methionine treatments were reported to increase DNA methylation 1.22-fold of controls after 8 weeks of coexposure.

In the 44-week study, the authors report that foci and hepatocellular adenomas were found. However, the authors do not report the incidences of these lesions in their study groups (how many of the treated animals developed lesions). As noted above, the numbers of animals in these groups varied widely between treatments (e.g., $n = 36$ for DCA and coexposure to 8.0 g/kg methionine but only $n = 16$ for 8 g/kg methionine treatment alone). Although reporting unscheduled deaths in the 8.0 g/kg methionine and DCA coexposure groups, the authors did not indicate whether these mortalities occurred in the 44-week or 8-week study groups. Multiplicities of foci and adenoma data were presented. DCA was reported to induce 2.42 ± 0.38 foci/mouse and 1.28 ± 0.31 adenomas/mouse (m ± SE) after 44 weeks of treatment. The DCA-induced foci and adenomas were reported to stain as eosinophilic with "relatively large hepatocytes and nuclei." The authors did not present data on the percent of foci and adenomas that were eosinophilic using this paradigm. The addition of 4.0 or 8.0 g/kg methionine to the AIN-76A diet was reported to reduce the number of DCA-induced adenomas/mouse to 0.167 ± 0.093 and 0.028 ± 0.028, respectively. However, the addition of 4.0 g/kg methionine to the DCA treatment was reported to increase the number of foci/mouse (3.4 ± 0.46 foci/mouse). The addition of 8.0 g/kg methionine to the DCA treatment was reported to yield 0.94 ± 0.24 foci/mouse. There were no foci or tumors in the 16 mice that received either the control diet or the 8.0 g/kg methionine treatment without DCA. The authors did not report whether methionine treatment had an effect on the tincture of the foci or adenomas induced by DCA.

Therefore, a very high level of methionine supplementation to an AIN-760A diet, was shown to affect the number of foci and adenomas, i.e., decrease them, after 44 weeks of coexposure to very high exposure concentration of DCA. However, a lower level of methionine coexposure increased the incidence of foci at the same concentration of DCA. Methionine treatment alone at the 8 g/kg level was reported to increase liver weight, decrease lauroyl-CoA activity and to increase DNA methylation. No histopathology was given by the authors to describe the effects of methionine alone. Coexposure of methionine with 3.2 g/L DCA was reported to decrease by ~25% DCA-induced glycogen accumulation and increase mortality, but not to have much of an effect on peroxisome enzyme activity (which was not elevated by more than 33% over control for DCA exposure alone). The authors suggested that their data indicate that methionine treatment slowed the progression of foci to tumors. Whether, these results would be similar for lower concentrations of DCA and lower concentrations of methionine that were administered to mice for longer durations of exposure, cannot be ascertained from these data. It is possible that in a longer-term study, the number of tumors would be similar. Whether, methionine treatment coexposure had an effect on the phenotype of foci and tumors was not presented by the authors in this study. Such data would have been valuable to discern if methionine coexposure at the 4.0 mg/kg level that resulted in an increase in DCA-induce foci, resulted in foci of a differing phenotype or a more heterogeneous composition than DCA treatment alone.

E.2.3.2.13. *DeAngelo et al., 2008.* In this study, neutralized TCA was administered in drinking water to male B6C3 F1 mice (28−30 days old) in three studies. In the first study control animals received 2 g/L sodium chloride while those in the second study were given 1.5 g/L neutralized acetic acid (HAC) to account for any taste aversion to TCA dosing solutions. In a third study deionized water served as the control. No differences in water uptake were reported. Mean initial weights were reported to not differ between the treatment groups (19.5 ± 2.5 g – 21.4 ± 1.6 g or ~10% difference). The first study was reported to be conducted at the U.S. EPA laboratory in Cincinnati, OH in which mice were exposed to 2 g/L sodium chloride, or 0.05, 0.5, or 5 g/L TCA in drinking water for 60 weeks. There were 5 animals at each concentration that were sacrificed at 4, 15, 31, and 45 weeks with 30 animals sacrificed at 60 weeks of exposure. There were 3 unscheduled deaths in the 0.05 g/L TCA group leaving 27 mice at final necropsy. For the other exposure groups there were 29 or 30 animals at final necropsy. In the second study, also conducted in the same laboratory, mice were reported to be exposed to 1.5 g/L neutralized acetic acid or 4.5 g/L TCA for 104 weeks. Serial necropsies were conducted (5 animals per group) at 15, 30, and 45 weeks of exposure and on, 10 animals in the control group at 60 weeks. For this study, a total of 25 animals were sacrificed in interim necropsies in the 1.5 g/L HAC group and 15 in the 4.5 g/L TCA group. There were 7 unscheduled deaths in the HAC group and 12 in the 4.5 g/L TCA group leaving 25 animals at final necropsy and 30 animals in the final necropsy groups, respectively. Study 3 was conducted at the U.S. EPA laboratory in RTP NC. Mice were exposed to deionized water or 0.05 or 0.5 g/L TCA in the drinking water for 104 weeks with serial necropsies (n = 8 per group) conducted at 26, 52, and 78 weeks. There were 19−21 animals reported at interim sacrifices and 17 unscheduled deaths in the deionized water group, 24 unscheduled deaths in the 0.05 g/L TCA group, and 24 unscheduled deaths in the 0.5 g/L TCA group. This left 34 mice at final necropsy in the control group, 29 mice in the 0.05 g/L TCA group, and 27 mice in the 0.5 g/L group.

At necropsy, liver, kidneys, spleen and testes weights were reported to be taken and organs examined for gross lesions. Tissues were prepared for light microscopy and stained with H& E. At termination of the exposure periods, a complete rodent necropsy was reported to be performed. Representative blocks of tissue were examined only in 5 mice from the high dose and control group with the exception of gross lesions, liver, kidney, spleen and testis at interim and terminal sacrifices. If the number of any histopathologic lesions in a tissue was "significantly increased above that in control animals" then that tissue was reported to be examined in all TCA dose groups. For Study #3 a second contract pathologist reviewed 10% of the described hepatic lesions. No "major differences" were reported between the two pathologic diagnoses. The prevalence and multiplicity of hepatic tumors were reported to be derived by performing a histopathologic examination of surface lesions and four sections cut from each of four tissue blocks excised from each liver lobe. Tumor prevalence was reported to be calculated as the percentage of the animals with a neoplastic lesion compared to the number of animals examined. Tumor multiplicity was reported to be calculated by dividing the number of each lesion or combined adenomas and carcinomas by the number of animals examined. Preneoplastic large foci of cellular alteration were also observed over the course of the study.

The prevalence and severity of hepatocellular cytoplasmic alterations, inflammation, and necrosis were reported to be determined using a scale based on the amount of liver involved of 1 = minimal (occupying 25%), 2 = mild (occupying 25−50%), 3 = moderate (occupying 50−75%) and 4 = marked (occupying >75%). The only "significant change outside of the liver" was reported to be testicular degeneration. LDH was determined in arterial blood collected at 30 and 60 weeks (Study 1) and 4, 30, and 104 weeks (Study 2). Cyanide insensitive PCO was also reported to be measured. Five days prior to sacrifice, tritiated thymidine (Studies 1 and 2) or BrdU (Study 3) was administered via miniosmotic pumps and the number of hepatocyte nuclei with grain counts >6 were scored in 1,000 cells or chromogen pigment over nuclei (BrdU). The labeling index was calculated by dividing the number of labeled hepatocyte nuclei by total number of hepatocytes scored. Total neoplastic and preneoplastic lesions (multiplicity) were counted individually or combined (adenomas and carcinomas) for each animal. The analysis of tumor prevalence data were reported to include only those animals examined at the scheduled necropsies or animals surviving to Week 60 (Study 1) or longer than 78 weeks (Studies 2 and 3). The data from all the scheduled necropsies was combined for an overall test of treatment-related effect.

For Study #1 (60-week exposure) all TCA treated groups experienced a decrease in drinking water consumption with the decreases in drinking water for the 0.5 and 5 g/L TCA exposure groups reported as statistically significant by the authors. The water consumption in mL/kg-day was reported to be reduced by 11, 17, and 30% in the 0.05, 0.5, and 5 g/L TCA treated groups compared to 2 g/L NaCl control animals as measured by time-weighted mean daily water consumption measured over the study. The control value was reported to be 171 mL/kg/day. Although the 0.05 g/L exposure concentrations were not measured, the 0.5 and 5 g/L solutions were within 4% of target concentrations. The authors estimated that the mean daily doses were 0, 8 mg/kg, 68 mg/kg and 602 mg/kg per day. For the 102 week studies the mean water consumption with deionized water was reported to be 112 mL/kg/day and 132 mL/kg/day for control animals given 1.5 g/L HAC. Therefore, there appeared to be a 35% decrease in water consumption between the controls in Study #1 given 2 g/L NaCl and controls in a Study #3 given deionized water but conducted at a different laboratory. There appeared to be a 23% reduction in water consumption between animals given 2 g/L NaCl and those given 1.5 g/L HAC at the same laboratory (Study #2). As the concentrations of TCA were increased, there would be a corresponding increase in the amount of sodium hydroxide needed to neutralize the solutions and a corresponding increase in salts in the solution as well as TCA. The authors did not address nor discuss the differences in drinking water consumption between the differing control solutions between the studies. DeAngelo et al. (1999) reported mean drinking water consumption of 147 mL/kg/day in control mice of over 100 weeks and that the highest dose of DCA (3.5 g/L) reduced drinking water consumption by 26%. Carter et al. (1995) reported that DCA at 5 g/L to decrease drinking water consumption by 64 and 46% but 0.5 g/L DCA to not affect drinking water consumption. While reporting that Study #1 showed that increasing TCA concentration decreased drinking water consumption, the drinking water consumption in Studies #2 and #3 were similar between controls and TCA exposure groups with both being less than the control and low TCA concentration values reported in Study #1 (i.e., in Study #2 the 1.5 g/L HAC and 4.5 g/L TCA drinking water consumption was ~130 mL/kg/day and in Study #3 the drinking water consumption was ~112 mL/kg/day for the deionized water control and 0.05 g/L and 0.5 g/L TCA exposure groups). Thus, the drinking water concentrations for Study #3 was ~35% less than for the control values for Study #1 and was also ~25% less than for DeAngelo et al. (1999). The reasons for the apparently lower drinking water averages for Study #3 and the lack of effect of the addition of 0.5 g/L TCA that was reported in Study #1 and in other studies, was not discussed by the authors.

In Study #1, there was little difference between exposure groups (n = 5) noted for the final body weights (mean range of 27.6−28.1 g) in mice sacrificed after 4 weeks of exposure. However, absolute liver weight and percent liver/body weight ratios increased with TCA dose. The percent liver/body weight ratios were 5.7% ± 0.4%, 6.2% ± 0.3%, 6.6% ± 0.4%, and 7.7% ± 0.6% for the 2 g/L NaCl control, 0.05, 0.5, and 5 g/L TCA exposure groups, respectively. These represent 1.09-, 1.16-, and 1.35-fold of control levels that were statistically significant. At 15 weeks of exposure the fold increases in percent liver/body weight ratios were 1.14-, 1.16-, and 1.47-fold of controls for 0.05, 0.5, and 5 g/L TCA. At 31 weeks of exposure the fold increases in percent liver/body weight ratios were 0.98-, 1.09-, and 1.59-fold of controls for 0.05, 0.5, and 5 g/L TCA. At 45 weeks of exposure the fold increases in percent liver/body weight ratios were 1.13-, 1.45-, and 1.98-fold of controls for 0.05, 0.5, and 5 g/L TCA. At 60 weeks of exposure the percent liver/body weight ratios were 0.94-, 1.25-, 1.60-fold of controls for 0.05, 0.5, and 5 g/L TCA. Thus, the range of increase at the lowest level of TCA exposure (i.e., 0.05 g/L) was 0.94- to 1.14-fold of controls. These data consistently show TCA-induced increases in liver weight from 4 to 60 weeks of the study that were dose-related. For the 0.5 g/L exposure group, the magnitude of the increase compared to control was reported to be about the same between weeks 4 and 30 with the highest increase reported to be at Week 45 (1.45-fold of control). In regard to the correspondence with magnitude of difference in dose of TCA and liver weight increase, there was ~2-fold increase in liver weight gain corresponding to 10-fold increases in TCA concentration at 4 weeks of exposure. For the 4 and 15-week exposures there was ~3.3- and 3.9-fold difference in liver weight that corresponded to a 100-fold difference in exposure concentration of TCA (i.e., 0.05 vs. 5.0 g/L TCA).

The small number of animals examined, n = 5, limit the power of the study to determine the change in percent liver/body weight up to 45 weeks, especially at the lowest dose. However, the 0.05 g/L TCA exposure groups at 4 week and 15 weeks were reported to significantly increase percent liver/body weight ratios. The percent liver/body weight ratios for all of the treatment groups and the ability to detect significant changes were affected by changes in final body weight and changing numbers of animals. After 4 to 30 weeks of exposure, the final body weights of mice increased in control animals but were within 11% of each other between weeks 31 and 60. The percent liver/body weight ratios in controls decreased from 4 to 31 weeks and were slightly elevated by 60 weeks compared to the 31-week level. Although control values were changing, there appeared to be no difference between control values and treated values in final body weight for any duration of exposure with the exception of the 5 g/L TCA exposure group after 60 weeks of exposure, which was decreased by ~15%. At the 31-week and 60-week exposure durations, the 0.05 g/L TCA groups did not have increased percent liver/body weight ratios over controls.

In Study #2, conducted in the same laboratory but with a 1.5 g/L HAC solution used for control groups, there was less than 5% difference in final body weights between control mice give HAC and those treated with 4.5 g/L TCA up to 45 weeks. However, final body weight was reduced by TCA treatment by 104 weeks by ~15%. Between the interim sacrifices of 15, 30, and 45 weeks, the percent liver/body weight ratios in control mice were similar at 15 and 45 weeks (~4.8%) but greater in the 30-week control group (5.3% or ~10% greater than other interim control groups). The TCA-induced increases in body weight were 1.60-, 1.40-, and 1.79-fold of control for the 15, 30, and 45 week groups exposed to 4.5 g/L TCA in Study #2. The smaller magnitude of TCA-induced liver weight increase at 30-weeks that that for 15 and 45 weeks, was a reflection of the increased percent liver/body weight ratio reported for the HAC control at that time point.

Comparisons can be made between Study #1 and Study #2 for 4.5 g/L or 5.0 g/L TCA exposure levels and controls for 15, 30/31 and 45 weeks of exposure to ascertain the consistency of response from the same laboratory. Although the two studies had differing control solutions and reported different drinking water consumption overall, they were exposing the TCA groups to almost the same concentration of TCA in the same buffered solutions for the same periods of time with the same number of mice per group. Between Study #1 and Study #2, there were consistent percent liver/body weight ratios induced by either 5.0 g/L TCA and 4.5 g/L TCA at weeks 15 and 30/31 (i.e., within 3% of each other). The percent liver/body ratios for these exposure groups ranged from 7.3−7.7% between weeks 15 and 30/31 for the ~5.0 g/L TCA exposure in both studies. Final body weights were within 10%. While the percent liver/body weight ratios induced by ~5.0 g/L TCA were similar, the magnitude of increase in comparison to the controls was 1.47- and 1.59-fold of control for Study #1, and 1.60- and 1.40-fold of control for Study #2 after 15 and 30/31 weeks of exposure, respectively. At 45 weeks, the percent liver/body weight ratios were within 11% of each other (9.4 vs. 8.4%) and final body weights were within 2% of each for this exposure concentration between the two studies giving a 1.98- and 1.79-fold of control percent liver/body weight, respectively. Thus, the apparent magnitude of TCA-induced increase in percent liver/body weight was affected by control values used as the basis for comparison. The percent liver/body weights reported for either 4.5 g/L TCA or 5.0 g/L TCA exposure groups for weeks 15 and 30/31 was similar between the two studies conducted in the same laboratory.

Study #3 was conducted in a separate laboratory, interim sacrifice times were not the same as for Study #1, the number of animals examined differed ($n = 5$ for Study #1 and $n = 8$ for Study #3), and control animals studied for comparative purposes were given different drinking water solutions (deionized water vs. 2 g/L NaCl). Most importantly the body weights reported at 52 weeks was much grated than that reported at 45 weeks for Studies #1 and #2. However, a comparison of TCA-induced liver weight gain and the effects of final body weight can be made between the 0.05 and 0.5 g/L TCA exposure groups at 30 weeks (Study #1) and 26 weeks (Study #3), at 45 weeks and 60 weeks (Study #1), and 52 weeks (Study #3). At 31 weeks there was <2% difference in mean final body weights between control and the two TCA-treatment groups in Study #1. There was also little difference between the TCA-treated groups at week in Study #3 at Week 26 and the TCA treatment groups in at Week 31 in Study #1 (i.e., range of 42.6−43.5 g for 0.05 and 0.5 g/L TCA treatments in Studies #1 and #3). However, in Study #3, the control value was 12% lower than that of Study #1 for mean final body weight. Based on final body weights, there would be an expectation of similar results between the two studies at the 26 and 30 week time points. At the 45 week (Study #1), and 52-week (Study #3), and 60-week (Study #1) durations of exposure, the mean final body weights varied little between their corresponding control groups at each sacrifice time (less than 4% variation between control and TCA-treated groups). However, there was variation in mean final body weights between the differing sacrifice times. Control and TCA-treated groups were reported to have lower mean final body weights at 45 weeks of exposure in Study #1 than at either 30 weeks or at 60 weeks. The 45-week mean final body weights in Study #1 were also reported to be lower than those at 52 weeks in Study #3. Control mean body weight values were 28% higher at 52 weeks in Study #3 than 45 weeks in Study #1 and 15% higher for 60 weeks in Study #1. In essence, for Study #1 mean final body weights went down between 31 and 45 weeks of exposure and then went back up at 60 weeks of exposure for control mice (~43, ~40, and ~44 g for 31, 45, and 60 weeks, respectively) as well as for both TCA concentrations. However, for Study #3 final mean body weights went up between 26 and 52 weeks of exposure for control mice (~39 vs. ~51 g) and for both TCA concentrations. While for Study #1 the percent liver/body weight ratios were 0.98- and 1.09-fold of control at 31 weeks of exposure, at Week 45 the ratios were 1.13- and 1.45-fold of control, and at Week 60 they were 0.94- and 1.25-fold of controls for the 0.05 and 0.5 g/L TCA exposure levels, respectively. For Study #3, the pattern differed than that of Study #1. There was a 1.07- and 1.18-fold of control percent liver/body weight for 26 weeks but a 0.92- and 1.04-fold of control percent liver/body weight change at 52 weeks of exposure at 0.05 and 0.5 g/L TCA exposure, respectively.

Thus, there appeared to be differences in control and the treatment groups at the 26 week sacrifice groups in Study #3 that was not apparent at the 52-week sacrifice time. Overall, the final body weights appeared to be similar between controls and TCA treatment groups at the 52-week sacrifice time in Study #3 and at the 31-, 45-, and 60-week sacrifice times in Study #1. However, although consistent within sacrifice times, the final body weights differed between the various sacrifice times in Studies #1 and #3. The patterns of percent liver/body weight at differing and similar sacrifice times appeared to differ between the Study #1 and Study #3 at the same concentrations of TCA. The largest difference appeared to be between Week 45 group in Study #1 and Week 52 group in Study #3 where both concentrations of TCA were reported to induce increases in percent liver/body weight in one study but to have little difference in the other. The differences in mean final body weights between these two sacrifice times were also the largest although control and TCA-treatment groups had little difference on this parameter. Similar to the work of Kjellstrand et al with TCE (Kjellstrand et al., 1983a), the groups with the lower body weight appeared to have the greatest response in liver weight increase.

These data illustrate the variability in findings of percent liver weight induction between laboratories, studies, choice of controls solutions, and the affects of final body weights on this parameter. They also illustrate the limitations for determining either the magnitude or pattern of liver weight increases using a small number of test animals. As animals age the size of their liver changes but also during the latter parts of the lifespan, foci and spontaneously occurring liver tumors can affect liver weight. The results of Study #1 show a consistent dose-response in TCA liver weight increases at 4 and 15 week time periods over a range of concentration from 0.05 g/L to 5 g/L TCA.

In regard to non-neoplastic pathological changes the authors reported that

> Increased incidences and severity of centrilobular cytoplasmic alterations, inflammation, and necrosis were the only nonproliferative changes seen in livers of animals exposed to TCA for 60 weeks (Tables 7-9; Study 1. Incidences were between 21 and 93%; severity ranged from minimal to mild; and some lesions were transient. Centrilobular cytoplasmic alterations (Table 7) were the most prominent nonproliferative lesion. The incidence and severity were dose related and significantly increased at all TCA concentrations. Centrilobular alterations are a low-grade degeneration of the hepatocytes characterized by an intense eosinophilic cytoplasm with deep basophilic granularity (microsomes) and slight hepatomegaly. The distribution ranged from centrilobular to diffuse. The incidence of inflammation was increased significantly in the 5 g/L TCA treatment group (Table 8), but was significantly lower in the 0.05- and 0.5 g/L groups between 31 and 45 weeks, but abated by 60 weeks. There was a significant dose-related trend, but a significant increase in severity was only found at 5 g/L. No

alteration in the severity of this lesion was observed. The occurrence and severity of nonproliferative lesions in animals exposed to 0.5 and 4.5 g/L TCA for 104 weeks were similar to those observed at 60 weeks (data not shown). No pathology outside the liver was observed except for a significant dose-related trend and incidence of testicular tubular degeneration at 0.5 and 5 g/L TCA.

The results shown in Table 7 by the authors for the 60-week TCA-exposed mice did not show a dose-response for either incidence or severity of centrilobular cytoplasmic alterations. They reported a 7, 48, 21, and 93% incidence and a 0.10 ± 0.40, 0.70 ± 0.82, 0.34 ± 0.72 and 1.60 ± 0.62 mean severity score for control, 0.05, 0.5, and 5.0 g/L TCA exposure groups, respectively. Thus, for control, 0.05 and 0.5 g/L TCA exposure there was less than minimal (i.e., score of 1 or occupying less than 25% of the microscopic field) severity of this finding for the 27 to 30 mice examined in each group. Only slight hepatomegaly is noted by the authors to be included in their description of the centrilobular cytoplasmic alteration. Interestingly, the elevation of this parameter for both incidence and severity in the 0.05 g/L TCA exposed group compared to 0.5 g/L exposure group did not correspond to an increase in percent liver/body weight for this same exposure group. While the percent liver/body weight ratio was 32% higher, the incidence and severity of this lesion were reported to be half that in the 0.5 versus 0.05 g/L exposure groups after 60 days of TCA exposure. Thus, TCA-induced hepatomegaly did not appear to be associated with this centrilobular cytoplasmic change. Similarly the incidence of hepatic inflammation was reported to be 10, 0, 7, and 24% and severity, 0.11 ± 0.40, 0.09 ± 0.30, 0.12 ± 0.33, and 0.29 ± 0.48 for control, 0.05, 0.5, and 5.0 g/L TCA exposure groups, respectively. Thus, at no TCA exposure concentration was the incidence more than 24% and the severity was considerably less than minimal. The reported results for hepatic necrosis were pooled from data from the 5 mice exposed for either 30 or 45 weeks (n = 10 total). No incidences of necrosis were reported for either control or 0.05 g/L TCA exposed mice. At 0.5 g/L TCA 3/10 mice were reported to have necrosis but at a severity level of 0.50 ± 0.97. At 5.0 g/L TCA 5/10 mice were reported to have necrosis but at a severity level of 1.30 ± 1.49. The limitations of the small number of animals pooled in these data are obvious. However, there does not appear to be much more than minimal necrosis at the highest dose of TCA between 30 and 45 weeks and this response is reported by the authors to be transient.

Serum LDH activity was reported by the authors for 31 and 60 week TCA exposures in Study #1. They state that

> There was a dose-related trend at 31 weeks; serum LDH was significantly increased at 0.5 and 5 g/L TCA (161 ± 39 and 190 ± 44, respectively vs. 100 ± 28 IU for the control). LDH activity returned to control levels at 60 weeks.

Similarly, elevated LDH levels were observed at early time periods for 0.5 and 4.5 g/L TCA during the 104 week exposure (data not shown: Studies 2 and 3).

The data presented by the author for Study #1 are from 5 animals/group for the 30-week results and 30 animals/group for the 60-week results. Of interest is for the 60-week data, there appears to be 50% decreased in LDH activity at 0.05 and ~25% decrease in LDH activity at 0.5 g/L TCA treatment with the LDH level reported to be the same as control for the 5 g/L TCA exposure group. For the 31-week data, in which only 5 animals were tested in each treatment group, there appeared to be a slight increase at the 0.5 g/L (60% increase over control) and 5 g/L (90% increase over control) treatment groups. The data for necrosis detected by light microscopy and by LDH level is consistent with no changes from control detected at the 0.05 g/L TCA treatment group and less than minimal necrosis of on a 60% increase in LDH level over control reported for 0.5 g/L TCA treatment. Even at the highest dose of 5.0 g/L TCA there is still little necrosis or LDH release reported over control.

Data for testicular tubular degeneration was reported for Study #1 after 60-weeks of TCA exposure. The incidence of testicular tubular degeneration was reported to be 7, 0, 14, and 21% for mice exposed to 2.0 g/L NaCl, 0.05, 0.5, and 5.0 g/L TCA. The severity of the lesions was reported to be 0.10 ± 0.40, 0, 0.17 ± 0.47, and 0.21 ± 0.41 with a significant trend with dose reported by the authors for severity and for the 0.5 and 5 g/L treatment groups to be significantly increased over control incidence levels. Of note, similar to the percent liver/body weight ratios and hepatic inflammation values for this data set, the values for testicular tubular degeneration were slightly higher in control mice than 0.05 g/L TCA exposed mice. In regard to mean severity levels for testicular degeneration, although still minimal, there was little difference between the results for reported for the 0.5 g/L TCA and 5.0 g/L TCA exposed mice.

In regard to peroxisome proliferation, liver PCO activity was presented for up to 60 weeks (Study #1) and 104 weeks (Study #2). Similar to the data for LDH activity, ~30 animals were examined at the 60-week time point but only 5 animals per exposure group were examined for 4-, 15-, 31-, and 45-week results. The data are presented in a figure and in some instances hard to determine the magnitude of change. Similar to other reports, the baseline level of PCO activity was variable between control groups and ranged 2.7-fold (~1.49 to 4.06 nmol NAD reduced/min/mg protein given by the authors). There appeared to be little change in PCO activity between the 0.05 g/L TCA exposure and control levels for up to 45 weeks of exposure (i.e., the groups with $n = 5$) in Study #1. For the 60-week group the 0.05 g/L TCA group PCO activity was ~1.7-fold of control but was not statistically significant. For the 0.5 g/L TCA treatment groups, the increase ranged from ~1.3- to 2.7-fold of control after 4-, 15-, 31-, and 45-weeks of exposure with the largest differences reported at 4 and 60 weeks (i.e., 2.2- and 2.7-fold of control, respectively). For the 5.0 g/L TCA exposure groups, the increase ranged from ~3.2- to ~5.7-fold of control after 4, 15, 31, and 45 weeks of exposure. While the data at 60-weeks had the most animals examined (~30 vs. 5) with ~1.7-, 2.7-, and 4.5-fold of control PCO activity, at this time period the authors report the occurrence of tumors had already occurred. At the earlier time points of 4 and 15 weeks, there was a difference in the magnitude TCA-induced increase in PCO activity. As displayed graphically, at 4 weeks the PCO increase was ~1.3-, 2.4-, and 5.3-fold of control for 0.05, 0.5, and 5.0 g/L TCA, respectively, while at 15 weeks, the PCO levels were decreased by 5%, increased to 1.3-fold, and increased to 3.2-fold of control with only the 5.0 g/L treatment group difference to be statistically significant.

For Study #2 the authors present a figure (Figure #4) that states that PCO values were given for mice given HAC or 4.5 g/L TCA for 4−60 weeks. However, the data presented in #4 appears to be for 15-, 30-, 45- and 104-week exposures. The number of mice is not given in the figure but the methods section states that serial section were conducted on 5 mice/group for these interim sacrifice periods. The number of mice examined for PCO activity at 104 weeks was not given by the authors but the number of mice at final sacrifice was given as 25. The levels of PCO in the control tissues varied by ~33% for weeks 15 to 45 but there was a ~5-fold difference between the level reported at 104 weeks and that for the earlier time periods in control mice shown in the figures (~2.23 vs. 0.41 nmol NAD reduced/min/mg protein as given by the authors). The increase over control induced by 4.5 g/L TCA in Study #2 was shown to be ~6.9-, 4.8-, 3.6-, and 19-fold of controls for 15, 30, 45 and 104 weeks, respectively.

Therefore, at a comparable level of TCA exposure (~5.0 g/L), number of mice examined ($n = 5$), and durations of exposure (15, 30, and 45 weeks), the increase in PCO activity induced by ~5.0 g/L TCA varied between 3.2- to 5.7-fold of control in Study #1 and between 3.6- to 6.9-fold of control in Study #2. There was not a consistent pattern between the two studies in regard to level of PCO induction from ~5 g/L TCA and duration of exposure. The lowest TCA-induced PCO activity increase was recorded at 15 weeks in Study #1 (i.e., 3.2-fold of control) and highest PCO activity increase was recorded at 15 weeks in Study #2 (i.e., 6.9-fold of control). No PCO data were reported for data in Study #3 with the exception of the authors stating that "PCO activity was significantly elevated for the 0.5 g/L TCA exposure over the 104 weeks (study 3). The extent of the increases was similar to those measured for 0.5 g/L TCA (200-375%: data not shown) in Study 1." No other details are given for PCO activity in Study #3.

Hepatocyte proliferation was reported by the authors to be assessed by either incorporation of tritiated thymidine (Studies #1 and #2) or BrdU (Study #3) into hepatocyte nuclei. As noted previously, these techniques measure DNA synthesis and not necessarily hepatocyte proliferation. The authors did not report if specific areas of the liver were analyzed by autoradiographs or how many autoradiographs were examined in the analyses they conducted. For later time points of examination (60−104 weeks) the authors did not indicate whether hepatocytes in foci or adenomas were excluded from DNA synthesis reports. The authors present data for what are clearly, 31, 45, and 60 week exposure for Study #1 as the percent tritiated thymidine labeled nuclei. An early time point that appears to be 8 weeks is also given. However, for Study #1 only 4 week and 15 week durations were tested so it cannot be established what time period the earlier time point represents. What is very apparent from the data presented for Study #1 is that the baseline level of tritiated thymidine incorporation was relatively high and highly variable for the 5 animals examined (~8% of hepatocytes were labeled). There did not appear to be an apparent pattern of TCA treatment groups at this timepoint with the 0.05 and 5.0 g/L TCA groups having a similar percentage of labeled hepatocytes and for 0.5 g/L TCA reported to have a 60% reduction in labeled hepatocytes. After 31 weeks of exposure the control values were reported to be 2% of hepatocytes labeled. The authors report that only the 5.0 g/L TCA group had a statistically significant increase of control and was elevated to ~6% of hepatocytes. The two lower doses of TCA had similar reported incidences of labeled hepatocytes of 4.5% that were not reported to be statistically significant. For the 45-week exposure period in Study #1, the control value was reported to be 1.2% with only the 5.0 g/L TCA value reported to be statistically significantly increased at 3.2% and the other two TCA groups to be similar to control. Finally, for the 60 week group from Study #1, the control value was reported to be 0.6% of hepatocytes labeled and the only the 0.5 g/L TCA dose reported to be statistically significantly increased over control at 3.2%. What is clear from this study is that the control value for the unidentified early time point is much higher than the other values. There should not be such a large difference in mature mice nor such a high level. The difference in control values between the earlier time point and the 31-week time point was 4-fold. The difference between the earlier time point and the 45-week time point was ~7-fold. There did not appear to be an increase in hepatocyte tritiated thymidine labeling due to any concentration of TCA at the early unidentified time point (~Week 10 from the figure) from Study #1. There was no dose-response apparent for the other study periods and the percent of hepatocytes labeled were 3% or less. These results indicated DNA synthesis was not increased by 10−60 week exposures to TCA exposure that induced increased liver tumor response.

For Study #2 results were reported for tritiated thymidine incorporation into hepatocytes in a figure that was labeled as 4.5 g/L TCA and control tissue for 104 weeks but showed data for 15, 30, and 45 weeks of exposure. Of note is that the control values for this study were much lower than that reported for Study #1. The percent of hepatocytes labeled with tritiated thymidine was reported to be ~2% for the 15 week exposure period and less than 1% for the 30- and 45-week exposure periods. For the 4.5 g/L TCA exposures the percent hepatocytes labeled with tritiated thymidine were ~2−4% at all time points with only the 45 week period identified by the authors as statistically significant.

For Study #3, rather than tritiated thymidine, BrdU was used as a measure of DNA synthesis. The results are presented in Figure #8 of the report in which the 0.5 g/L TCA concentration is mislabeled as 0 g/L and the figure is mislabeled as having a duration of 104 weeks but the data are presented for 26, 52, and 78 weeks of exposure. The percent of hepatocytes at 26 weeks was reported to be ~1−2% for the control, 0.05 and 0.5 g/L TCA groups. At 52 weeks the control value was ~1% the 0.05 g/L TCA value was less than 0.1% and the 0.5 g/L TCA value was ~3.5% but not statistically significant. At 78 weeks of exposure the control value was reported to be ~0.2% with only the 0.05 g/L TCA group having a statistically significant increase over control.

From these data, the estimated control values for DNA synthesis at similar time points of exposure ranged from 0.4 to 2% at 26−31 weeks and ~0.1 to 1.2% at 45-52 weeks. The results for Study #1 and #2 were inconsistent in regard to the magnitude of tritiated thymidine incorporation but consistent in that there was a lot of variability in these measurements, not a consistent pattern with time that was TCA-dose related, and, even at the highest dose of TCA, did not indicate much of an increase in cell proliferation 15−45 weeks of exposure. Similarly the results for Studies #1 and #3 indicate that the two lower doses of TCA there were not generally statistically significant increases in DNA synthesis from 15−45 weeks of exposure although there was an increase in liver tumor response at later time points.

The authors reported that "all gross and microscopic histopathological alterations were consistent across the three studies." However, the histological descriptions that follow were focused on the liver for both neoplastic and non-neoplastic parameters. As stated above, only a few animals (*n* = 5) from the control and high TCA dose level were examined for lesions other than liver, kidneys, spleen and testes. Thus, whether other neoplastic lesions were induced by TCA exposure cannot be determined from this set of studies.

Study #1 was conducted for 60 weeks. Although of short duration and using 30 or less animals, the authors reported in the text that

> a significant trend with dose was found for liver cancer. The prevalence and multiplicity of adenomas (38%; 0.55 ± 0.15) or carcinoma (38%; 0.42 ± 0.11) were statistically significant at 602 mg/kg/day TCA compared to control (7%; 0.07 ± 0.05) [sic for both adenoma and carcinoma the same value was given, mean ± SD]. When either an adenoma or a carcinoma was present, statistical

significant was seen at both 5 g/L (55%; 1.00 ± 0.19) and 0.5 g/L (38%: 0.52 ± 0.14 TCA exposure groups compared to control (13%; 0.13 ± 0.06). No significant change in liver neoplasia were reported to be observed by the authors at 0.05 g/L TCA. Preneoplastic large foci of cellular alteration (24%) were seen in the 5 g/L TCA control compared to control.

Although not statically significant, there was an incidence of 15% adenoma in the 0.05 g/L TCA treatment group (n = 27) and a multiplicity of 0.15 ± 0.07 adenomas/mouse reported with both values being twice that of the values given for the controls (n = 30). The incidence and multiplicity for carcinomas was approximately the same for the 0.05 g/L TCA treatment group and the control group. Given the small number of animals examined, the study was limited in its ability to determine statistical significance for the lower TCA exposure level. The fold increases of incidence and multiplicity of adenomas at 60 weeks was 2.1-, 3.0-, and 5.4-fold of control incidence and 2.1-, 3.4-, and 7.9-fold of control multiplicity for 0.05, 0.5, and 5 g/L exposure to TCA. For multiplicity of adenomas and carcinomas combined there was a 1.46-, 4.0-, and 7.68-fold of control values. Analysis of tumor prevalence data for this study included only animals examined at scheduled necropsy. Since most animals survived until 60 weeks, most were included and a consistent time point for tumor incidence was reported.

There are significant discrepancies for reporting of data for tumor incidences in this report for the 104 week data. While the methods section and table describing the dose calculation and animal survival indicate that Study #3 control animals were administered deionized water and those from Study#2 were given HAC, Table 6 of the report gives 2 g/L NaCl as the control solution given for Study #2 and 1.5 g/L HAC for Study #3. A comparison of the descriptions of animal survival and tumor incidence and multiplicity between the results given in DeAngelo et al. (2008) and George et al. (2000) (see Table E-10) shows not only that the control data presented in DeAngelo et al. (2008) for Study #3 to be the same data as that presented by George et al. (2000) previously, but also indicates that rather than 1.5 g/L HAC, the tumor data presented in DeAngelo et al. (2008) is for mice exposed to deionized water. DeAngelo et al. (2008) did not report that these data were from a previous publication.

Table E-10. Comparison of descriptions of control data between George et al. (2000) and DeAngelo et al. (2008)

Descriptor	George et al., 2000	DeAngelo et al., 2008
Species	Mouse	Mouse
Strain	B6C3F1	B6C3F1
Gender	Male	Male
Age	28−30 days	28−30 days
Source	Charles River, Portage	Charles River, Portage
Mean initial body wt	19.5 ± 2.5 g	19.5 ± 2.5 g
Water consumption	111.7 mL/kg/day	112 mL/kg/day
Laboratory	RTP NC	RTP NC
# Animals at start	72	72
# Animals at interim sac.	22	21
# Unscheduled deaths	16	17
# Animals at final sacrifice	34	34
# Animals for pathology	65	63
Adenoma incidence	21.40%	21%
Adenoma multiplicity	0.21 ± 0.06	0.21 ± 0.06
Carcinoma incidence	54.80%	55%
Carcinoma multiplicity	0.74 ± 0.12	0.74 ± 0.12

RTP NC = Research Triangle Park, North Carolina.

For Studies #2 and #3 tumor prevalence data were reported in the methods section of the report to include necropsies of animals that survived greater than 78 weeks and thus, included animals that were scheduled for necropsy but also those which were moribund and sacrificed at differing times. Thus, for the longer times of study, there was a mixture of exposure durations that included animals that were ill and sacrificed early and those that survived to the end of the study. Animals that were allowed to live for longer periods or who did not die before scheduled sacrifice times had a greater opportunity to develop tumors. However, animals that died early may have died from tumor-related causes. The mislabeling of the tumor data in DeAngelo et al. (2008) has effects on the interpretation of results for if the tumor results table was not mislabeled it would indicated 17 animals were included in the liver tumor analysis that were not included in the final necropsy and that the 7 unscheduled deaths could not account for the total number of "extra" mice included in the tumor analysis so some of the animals had to have come from interim sacrifice times (78 weeks or less) and that for Study #3 the data from 9 animals at terminal sacrifice were not used in the tumor analysis. Not only was the control data mislabeled for Study #3, but the control data were also apparently mislabeled for Study #2 as being 2.0 g/L NaCl rather than 1.5 g/L HAC. Of the 42 animals used for the tumor analysis in Study #3, only 34 were reported to have survived to interim sacrifice so that 8 animals were included from unscheduled deaths. However, the authors report that there were 17 unscheduled deaths in the study not all were included in the tumor analysis. The basis for the selection of the 8 animals for tumor analysis was not give by the authors.

Not only are the numbers of control animals used in the tumor analysis different between two studies (25 mice in Study #2 and 42 mice in Study #3), but the liver tumor results reported for Study #2 and Study #3 were very different. Of the 42 "control" mice examined from Study #3, the incidence and multiplicity of adenomas was reported to be 21% and 0.21 ± 0.06, respectively. For carcinomas, the incidence and multiplicity was reported to be 55% and 0.74 ± 0.12, respectively, and for the incidence and multiplicity of adenomas and carcinomas combined reported to be 64% and 0.93 ± 0.12, respectively. For the 25 mice reported by the authors for Study #2 to have been treated with "2.0g/L NaCl" but were probably exposed to 1.5 g/L HAC, the incidence and multiplicity of adenomas was 0%. For carcinomas, the incidence and multiplicity was reported to be 12% and 0.20 ± 0.12, respectively and for the incidence and multiplicity of adenomas and carcinomas combined to be 12% and 0.20 ± 0.12, respectively. Therefore, while ~64% the 42 control mice in Study #3 were reported to have adenomas and carcinomas, only 12% of the 25 mice were reported to have adenomas and carcinomas in Study #2 for 104-weeks.

While the effect of using fewer mice in one study versus the other will be to reduce the power of the study to detect a response, there are additional factors that raise questions regarding the tumor results. Not only were the tumor incidences were reported to be higher in control mice from Study #3 than Study #2, but the number of unscheduled deaths was reported to also be 2-fold higher. The age, gender, and strain of mouse were reported to be the same between Study #2 and #3 with only the vehicles differing and weight of the mice to be reported to be different. Although the study by George et al. (2000) describes the same control data set as for Study #3 as being for animals given deionized water, there is uncertainty as to the identity of the vehicle used for the tumor results reported for Study #3 and there are some discrepancies in reporting between the two studies. As discussed below in Section E.2.5, the differences in the weight of the mice between Studies #1, #2, and #3 is critical to the issue of differences in background tumor rate and hence interpretability of the study.

As noted by Leakey et al. (2003b), the greatest correlation with liver tumor incidence and body weight appears between the ages of 20 and 60 weeks in male mice. As reported in Section E.2.5, the mean 45-week body weight reported for control male B6C3F1 mice in the George et al. (2000) study, which is the same control data as DeAngelo et al. (2008) was ~50 g. This is a much greater body weight than reported for Study #1 at 45 weeks (i.e., 39.6 g) and for Study #2 at 45 weeks (i.e., 39.4 g). Using probability curves presented by Leakey et al. (2003b), the large background rate of 64% of combined adenomas and carcinomas for Study #3 is in the range predicted for such a large body weight (i.e., ~65%). Such a high background incidence compromises a 2-year bioassay as it prevents demonstration of a positive dose-response relationship. Thus, Study #3 of DeAngelo et al. (2008) is not comparable to the results in Study #1 and #2 for the determination of the dose-response for TCA.

The accurate determination of the background liver tumor rate is very important in determining a treatment related effect. The very large background level of tumor incidence reported for Study #3 makes the detection of a TCA-related change in tumor incidence at low exposure levels very difficult to determine. Issues also arise as to what the source of the tumor data were in the TCA-treatment and control groups in Study #3. While 29 mice exposed to 0.05 g/L TCA were reported to have been examined at terminal sacrifice, 35 mice were used for liver tumor analysis. Similarly, while 27 mice exposed to 0.5 g/L TCA were reported to have been examined at terminal sacrifice, 37 mice were used for tumor analysis. Finally, for the 42 control animals examined for tumor pathology in the control group, 34 were examined at terminal sacrifice. Clearly more animals were included in the analyses of tumor incidence and multiplicity than were sacrificed at the end of the experiment. What effect differential addition of the results from mice not sacrificed at 104 weeks and the selection bias that may have resulted from their inclusion on these results cannot be determined. Not only were the background levels of tumors reported to be increased in the control animals in Study #3 compared to Study #2 at 104 weeks, but the rate of unscheduled deaths was doubled. This is also an expected consequence of using much larger mice (Leakey et al., 2003b).

For the 35 mice examined after 0.05 g/L TCA in Study #3, the incidence and multiplicity of adenomas was reported to be 23% and 0.34 ± 0.12, respectively. For carcinomas, the incidence and multiplicity was reported to be 40% and 0.71 ± 0.19, respectively, and for the incidence and multiplicity of adenomas and carcinomas combined reported to be 57% and 1.11 ± 0.21, respectively. For the 37 mice examined after 0.5 g/L TCA in Study #3, the incidence and multiplicity of adenomas was reported to be 51% and 0.78 ± 0.15, respectively. For carcinomas, the incidence and multiplicity was reported to be 78% and 1.46 ± 0.21, respectively, and for the incidence and multiplicity of adenomas and carcinomas combined reported to be 87% and 2.14 ± 0.26, respectively. Thus, at 0.5 g/L TCA the results presented for this study for the "104 week" liver tumor data were significantly increased over the reported control values. However, these results are identical to those reported in Study #3 for a 10-fold higher concentration of TCA (4.5 g/L TCA) for the same 104 weeks of exposure but in the much larger mice. Of the 36 animals exposed to 4.5 g/L TCA in Study #2 and included in the tumor analysis, 30 animals were reported to be examined at 104 weeks. The incidence and multiplicity of adenomas was reported to be 59% and 0.61 ± 0.16, respectively. For carcinomas, the incidence and multiplicity was reported to be 78% and 1.50 ± 0.22, respectively, and for the incidence and multiplicity of adenomas and carcinomas combined reported to be 89% and 2.11 ± 0.25, respectively.

The importance of selection and determination of the control values for comparative purposes of tumor induction are obvious from these data. The very large difference in control values between Study #2 and Study #3 is the determinant of the magnitude of the dose response for TCA after 104 weeks of exposure. The tumor response for 0.5 and 4.5 g/L TCA exposure between the two experiments was identical. Therefore, only the background tumor rate determined the magnitude of the response to treatment. If a similar control values (i.e., a historical control value) were used in these experiments, there would appear to be no difference in TCA-tumor response between 0.5 and 4.5 g/L TCA at 104 weeks of exposure. DeAngelo et al. (1999) report for male B6C3F1 mice exposed only water for 79 to 100 weeks the incidence of carcinomas to be 26% and multiplicity to be 0.28 lesions/mouse. For 100-week data, the incidence and prevalence of adenomas was reported to be 10% and 0.12 ± 0.05 and for carcinomas to be 26% and 0.28 ± 0.07. Issues with reporting for that study have already been discussed in Section E.2.3.2.5. However, the data for DeAngelo et al. (1999) are more consistent with the control data for "1.5 g/L HAC" for Study #2 in which there were 0% adenomas and 12% carcinomas with a multiplicity of 0.20 ± 0.12, than for the control data for Study #3 in which 64% of the control mice were reported to have adenomas and carcinomas and the multiplicity was 0.93 ± 0.12. If either the control data from DeAngelo et al. (1999) or Study #2 were used for comparative purposes for the TCA-treatment results of Study #2 or #3, there would be a dose-response between 0.05 and 0.5 g/L TCA but no difference between 0.5 and 4.5 g/L TCA after 100 weeks of exposure. The tumor incidence would have peaked at ~90% in the 0.5 and 4.5 g/L TCA exposure groups. These results would be more consistent with the 60-week results in Study #1 in which 0.5 and 5 g/L TCA exposure groups already had incidences of 38 and 55% of adenomas and carcinomas combined, respectively, compared to the 13% control level. With increased time of exposure the differences between the two highest TCA exposure concentrations may diminish as tumor progression is allowed to proceed further.

However, the use of the larger and more tumor prone mice in Study #3 also increases the tumor incidence at the longer period of study.

The authors also presented data for multiplicity of combined adenomas or carcinomas for mice sacrificed at weeks 26, 52, and 78 for Study #3 (*n* = 8 per group). No indication of variability of response, incidence data, statistical significance, or data for adenomas versus carcinomas, or the incidence of adenomas was reported. The authors reported that "neoplastic lesions were first found in the control and 0.05 g/L TCA groups at 52 weeks. At 78 weeks, adenomas or carcinomas were found in all groups (0.29, 0.20, and 0.57 tumors/animals for control, 0.05 g/L TCA, and 0.5 g/L TCA, respectively)." Because no other data were presented at the 52 and 78 week time points in this study, these results cannot be compared to those presented for Study #1, which was conducted for 60 weeks. Of note, the results presented from Study #1 for 60 weeks of exposure to control, 0.05 g/L or 0.5 g/L TCA exposure in 27−30 mice show a 13, 15, and 38% incidence of hepatocellular adenomas and carcinomas and a multiplicity of 0.13 ± 0.06, 0.19 ± 0.09, and 0.52 ± 0.14, respectively. Both the incidence and multiplicity of adenomas were 2-fold higher in the 0.05 g/L TCA treatment group than for the control. However, the interim data presented by the authors from Study #3 for 52 weeks of exposure in only 8 mice per group gives a higher multiplicity of adenomas and carcinomas for control animals (~0.25) than for either 0.05 or 0.5 g/L TCA treatments. Again, comparisons between Study #2 and #3 are difficult due to difference in mouse weight.

Of note, there are no descriptions given in this report in regard to the phenotype of the tumors induced by TCA or for the liver tumors reported to occur spontaneously in control mice. Such information would have been of value as this study reports results for a range of TCA concentration and for 60 and 100 weeks of exposure. Insight could have been gained as to the effects of differing concentrations of TCA exposure, whether TCA-induced liver tumors had a similar phenotype as those occurring spontaneously, as well as information in regard to effects on tumor progression and heterogeneity.

Although only examining tissues from 5 mice from the control and high-dose groups only at 104 weeks at organ sites other than the liver, the authors report that

> neoplastic lesions at 104 weeks (Studies #2 and #3) at organ sites other than the liver were found in the lung, spleen, lymph nodes, duodenum (lymphosarcoma), seminal vesicles, skin, and thoracic cavity of control and treated animals. All were considered spontaneous for the male B6C3F1 mouse and did not exceed the tumor incidences when compared to a historical control database (Haseman 1984; NIEHS, 1998).

No data were shown. The limitations involved in examining only 5 animals in the control and high-dose groups, and the need to examine the concurrent control data in each experiment, especially given the large variation in liver tumor response between long-term studies carried out in the two different laboratories used for Study #2 and Study #3 using the same strain and gender of mouse, make assertions regarding extrahepatic carcinogenicity of TCA from this study impossible to support.

A key issue raised from this study is whether changes in any of the parameters measured in interim sacrifice periods before the appearance of liver tumors (i.e., 4−15 weeks) corresponded to the induction of liver tumors. The first obstacle for determining such a relationship is the experimental design of these studies in which only a full range of TCA concentrations is treated for 60 weeks of exposure with a small number of animals available for determination of a carcinogenic response (i.e., 30 animals or less in Study #1) and a very small number of animals (n = 5 group) examined for other parameters. Also as stated above, PCO activity was highly variable between controls and between treatment groups (e.g., the PCO activity for Study #1 and #2 at ~5 g/L exposure for 15 weeks). On the other hand, most of the animals that were examined at terminal sacrifice were also utilized for the tumor results without the differential deletion or addition of "extra" animals for the tumor analysis. For the 60-week data in Study #1 there appeared to be a consistent dose-related increase in the incidence and multiplicity of tumors after TCA exposure (Table E-11). The TCA-induced increases in liver tumor responses can be compared with both increased liver weight and PCO activity that were also reported to be increased with TCA dose as earlier events. Although the limitations of determining the exact magnitude of responses has already been discussed, as shown below, the incidence and multiplicity of adenomas show a dose-related increase at 60 weeks. However, the magnitude of differences in TCA concentrations was not similar to the magnitude of increased liver tumor induction by TCA after 60 weeks of exposure.

Table E-11. TCA-induced increases in liver tumor occurrence and other parameter over control after 60 weeks (Study #1)

Dose TCA g/L	Adenomas		Adenomas or carcinomas		% liver/body weight		PCO activity	
NaCl	Incidence 7%	Multiplicity 0.07	Incidence 13%	Multiplicity 0.13	4-week	15-week	4-week	15-week
0.05	15% (2.1-fold)	0.15 (2.1-fold)	15% (1.2-fold)	0.19(1.5-fold)	1.09-fold	1.14-fold	1.3-fold	1.0 -fold
0.5	21% (3.0-fold)	0.24 (3.4-fold)	38% (2.9-fold)	0.52 (4.0-fold)	1.16-fold	1.16-fold	2.4-fold	1.3-fold
5.0	38% (5.4-fold)	0.55 (7.9-fold)	55% (4.2-fold)	1.00 (7.7-fold)	1.35-fold	1.47-fold	5.3-fold	3.2-fold

First of all, the greater occurrence of TCA-induced increases in adenomas than carcinomas reported after 60 weeks of exposure would be expected for this abbreviated duration of exposure as they would be expected to occur earlier than carcinomas. For adenoma induction, there was a ~2-fold increase between the 0.05 g/L dose of TCA and the control group for incidence (7 vs. 15%) and multiplicity (0.07 vs. 0.15 tumors/animals). However, an additional 10-fold increase in TCA dose (0.5 g/L) only resulted in a reported 1.8-fold greater incidence (15 vs. 21%) and 2.2-fold increase in multiplicity (0.15 vs. 0.24 tumors/animal) of control adenoma levels. An additional 10-fold increase in dose (5.0 vs. 0.5 g/L TCA) resulted in a 2.2-fold increase in incidence (21 vs. 38%) and 2.9-fold increase in multiplicity (0.24 vs. 0.55 tumors/animal) of control adenoma levels. Thus, a 100-fold difference in TCA exposure concentration resulted in differences of 4-fold of control incidence and 6-fold of control multiplicity for adenomas. For adenomas or carcinomas combined (a parameter that included carcinomas for which only the two highest exposure levels of TCA were reported to increase incidence and multiplicity) the incidences were reported to be 13, 15, 38, and 55%, and the multiplicity reported to be 0.13, 0.19, 0.52, and 1.00 for control, 0.05, 0.5, and 5.0 g/L TCA at 60 weeks. For multiplicity of adenomas or carcinomas, the 0.05 g/L TCA exposure induced a 1.5-fold increase over control. An additional 10-fold increase in TCA (0.5 g/L) induced a 6-fold increase in tumors/animal. An additional 10-fold increase in TCA (5.0 vs. 0.5 g/L) induced an additional 2.2-fold increase in tumors/animal. Therefore, using combinations of adenomas or carcinomas, there was a 13-fold increase in multiplicity that corresponded with a 100-fold increase in dose.

The results for adenoma induction at 60 weeks of TCA exposure (i.e., ~2-fold increased incidences and 2- to 3-fold increases in multiplicity with 10-fold increases in TCA dose) are similar to the ~2-fold increase in liver weight gain resulting from 10-fold differences in dose reported at 4-weeks of exposure. For PCO activity there was a ~30% increase in PCO activity from control at 0.05 g/L TCA. A 10-fold increase in TCA exposure concentration (0.5 g/L) resulted in an additional ~5-fold increase in PCO activity. However, another 10-fold increase in TCA concentration (0.5 vs. 5 g/L) resulted in a 3-fold increase in PCO activity. The 100-fold increase in TCA dose (0.05 vs. 5 g/L TCA) was correlated with a 14-fold increase in PCO activity. For 15 weeks of TCA exposure there was no difference in 0.05 and control PCO activity and only a 30% difference between the 0.05 and 0.5 g/L TCA exposures. There was a 7-fold difference in PCO activity between the 0.5 and 5.0 g/L TCA exposure concentrations. The increases in PCO activity and liver weight data at 15-weeks did not fit the magnitude of increases in tumor multiplicity or incidence data at 60 weeks as well as did the 4-week data. However, the TCA-induced increase in tumors at 60 weeks (especially adenomas) seemed to correlate more closely with the magnitude of liver weight increase than for PCO activity at both 4 and 15 weeks.

In regard to Studies #1 and #2 there are consistent periods of study for percent liver/body weight with the consistency of the control values being a large factor in the magnitude of TCA-induced liver weight increases. As discussed above, there were differences in the magnitude of percent liver/body weight increase at the same concentration between the two studies (e.g., a 1.47-fold of control percent liver/body weight in the 5 g/L TCA exposed group in Study #1 and 1.60-fold of control in Study #2 at 15 weeks). For the two studies that had extended durations of exposure (Studies #2 and #3) the earliest time period for comparison of percent liver/body weight is 26 weeks (Study #3) and 30 weeks (Study #2). If those data sets (26 weeks for Study #3 and 30 weeks for Study #2) are combined, 0.05, 05, and 4.5 g/L TCA gives a percent liver body/weight increase of 1.07-, 1.18-, and 1.40-fold over concurrent control levels. Using this parameter, there appears to be a generally consistent pattern as that reported for Study #1 at weeks 4 and 15. Generally, a 10-fold increase in TCA exposure concentration resulted in ~2.5-fold increased in additional liver weight observed at ~30 weeks of exposure which correlated more closely with adenoma induction at 60 weeks than did changes in PCO activity. A similar comparison between Studies of longer duration (Studies #2 and #3) could not be made for PCO activity as data were not reported for Study #3.

For 104-week studies of TCA-tumor induction (Studies #2 and #3) the lower TCA exposure levels (0.05 and 0.5 g/L TCA) were assayed in a separate experiment and by a separate laboratory than the high dose (5.0 g/L TCA) and most importantly in larger more tumor prone mice. The total lack of similarity in background levels of tumors in Study #2 and #3, the differences in the number of animals included in the tumor analyses, and the low number of animals examined in the tumor analysis at 104 weeks (less than 30 for the TCA treatment groups) makes the determination of a dose-response TCA-induced liver tumor formation after 104-weeks of exposure problematic. The correlation of percent liver/body weight increases with incidence and multiplicity of liver tumors in Study #1 and the similarity of dose-response for early induction of percent liver/body weight gain between Study #1 suggest that there should be a similarity in tumor response. However, as noted above, the 104-week studies had very difference background rates of spontaneous tumors reported in the control mice between Study #2 and #3.

Table E-12, below, shows the incidence and multiplicity data for Studies #2 and #3 along with the control data for DeAngelo et al. (1999) for the same paradigm. It also provides an estimate of the magnitude of increase in liver tumor induction by TCA treatments if the control values from the DeAngelo et al. (1999) data set were used as the background tumor rate. As shown below, the background rates for Study #2 are more consistent with those of DeAngelo et al. (1999). Whereas there was a 2:1 ratio of multiplicity for adenomas and adenomas and carcinomas between 0.5 and 5.0 g/L TCA after 60 weeks of exposure, there was no difference in any of the data (i.e., adenoma, carcinoma, and combinations of adenoma and carcinoma incidence and multiplicity) for these exposure levels in Study #2 and #3 for 104 weeks. The difference in the incidences and multiplicities for all tumors was 2-fold between the 0.05 and 0.5 g/L TCA exposure groups in Study #2. These results are consistent with the two highest exposure levels reaching a plateau of response with a long enough duration of exposure (~90% of animals having liver tumors) and with the 2-fold difference in liver tumor induction between concentrations of TCA that differed by 10-fold, reported in Study #1.

If either the control values for Study #2 or the control values from DeAngelo et al. (1999) were used for as the background rate of spontaneous liver tumor formation, the magnitude of liver tumor induction by the 0.05 g/L TCA over control levels differs dramatically from that reported as control tumor rates in Study #3. To put the 64% incidence data for carcinomas and adenomas reported in DeAngelo et al. (2008) for the control group of Study #3 in context, other studies cited in this review for B6C3F1 mice show a much lower incidence in liver tumors in that: (1) the National Cancer Institute (NCI, 1976) study of TCE reports a colony control level of 6.5% for vehicle and 7.1% incidence of hepatocellular carcinomas for untreated male B6C3F1 mice (n = 70−77) at 78 weeks, (2) Herren-Freund et al. (1987) report a 9% incidence of adenomas in control male B6C3F1 mice with a multiplicity of 0.09 ± 0.06 and no carcinomas (n = 22) at 61 weeks, (3) NTP (1990) report an incidence of 14.6% adenomas and 16.6% carcinomas in male B6C3F1 mice after 103 weeks (n = 48), and (4) Maltoni et al. (1986) report that B6C3F1 male mice from the "NCI source" had a 1.1% incidence of "hepatoma" (carcinomas and adenomas) and those from "Charles River Co." had a 18.9% incidence of "hepatoma" during the entire lifetime of the mice (n = 90 per group). The importance of examining an adequate number of control or treated animals before confidence can be placed in those results in illustrated by Anna et al. (1994) in which at 76 weeks 3/10 control male B6C3F1 mice that were untreated and 2/10 control animals given corn oil were reported to have adenomas but from 76 to 134 weeks, 4/32 mice were reported to have adenomas (multiplicity of 0.13 ± 0.06) and 4/32 mice were reported to have carcinomas (multiplicity of 0.12 ± 0.06).

Table E-12. TCA-induced increases in liver tumor occurrence after 104 wks (Studies #2 and #3)

Dose TCA	Adenomas		Carcinomas		Adenomas or carcinomas	
	Incidence	Multiplicity	Incidence	Multiplicity	Incidence	Multiplicity
Study #3						
1.5 g/L HAC (H_20?)	21%	0.21	55%	0.74	64%	0.93
0.05 g/L TCA	23%	0.34	40%	0.71	57%	1.11
	(1.1-fold)	(1.6-fold)	(0.7-fold)	(1.0-fold)	(0.9-fold)	(1.2-fold)
0.5 g/L TCA	51%	0.78	78%	1.46	87%	2.14
	(2.4-fold)	(3.7-fold)	(1.4-fold)	(2.0-fold)	(1.4-fold)	(2.3-fold)
Study #2						
2.0 g/L NaCl (HAC?)	0%	0	12%	0.20	12%	0.20
4.5 g/L TCA	59%	0.61	78%	1.50	89%	2.14
	(?)	(?)	(6.5-fold)	(7.5-fold)	(7.4-fold)	(11-fold)
DeAngelo et al., 1999						
H_2O	10%	0.12	26%	0.28		
0.05 g/TCA (S #3)	(2.3-fold)	(2.8-fold)	(1.5-fold)	(2.5-fold)		
0.5 g/L TCA (S #3)	(5.1-fold)	(6.5-fold)	(3.0-fold)	(5.2-fold)		
5.0 g/L TCA (S #2)	(5.9-fold)	(6.5-fold)	(3.0-fold)	(5.4-fold)		

H_2O = water.

Using concurrent control values reported in Study #3, there is no increase in incidence of multiplicity of adenomas and carcinomas for the 0.05 g/L exposure group. However, compared to either the control data from DeAngelo et al. (1999) or the control data from Study #3, there is a ~2−3- or ~5-fold increased in incidence or multiplicity of liver tumors, respectively. Thus, trying to determine a correspondence with either liver weight increases or increases in PCO activity at earlier time points will be depend on the confidence placed in the concurrent control data reported in Study #3 in the 104 week studies. As noted previously, the use of larger tumor prone mice in Study #3 limits its usefulness to determine the dose-response for TCA.

The authors provide a regression analysis for “tumors/animal” or multiplicity as a percent of control values and PCO activity for the 60-week and 104-week data. Whether adenomas and carcinomas combined or individual tumor type were used was not stated. Also comparing PCO activity at the end of the experiments, when there was already a significant tumor response rather than at earlier time points, may not be useful as an indicator of PCO activity as a key event in tumorigenesis. A regression analysis of these data are difficult to interpret because of the dose spacing of these experiments as the control and 5 g/L exposure levels will basically determine the shape of the dose-response curve. The 0.05 and 0.5 g/L exposure groups in the regression were so close to the control value in comparison to the 5 g/L exposure, that the dose response will appear linear between control and the 5.0 g/L value with the two lowest doses not affecting the slope of the line (i.e., “leveraging” the regression). The value of this analysis is limited by (1) the use of tumor prone larger mice in Study #3 that had large background rates of tumors which make inappropriate the apparent combination of results from Studies #2 and #3 for the multiplicity as percentages of control values (2) the low and varying number of animals analyzed for PCO values and the variability in PCO control values (3) the appropriateness of using PCO values from later time points, and (4) the dose-spacing of the experiment.

Similarly, the authors report a regression analysis that compares “percent of hepatocellular neoplasia” which again is indicated by tumor multiplicity with TCA dose as represented by mg/kg/d. This regression analysis also is of limited value for the same reasons as that for PCO with added uncertainty as the exposure concentrations in drinking water have been converted to an internal dose and each study gave different levels of drinking water with one study showing a reduction of drinking water at the 5 g/L level. The authors attempt to identify a NOEL for tumorigenicity using tumor multiplicity and TCA dose. However, it is not an appropriate descriptor for these data, especially given that “statistical significance” of the tumor response is the determinant of the conclusions regarding a dose in which there is no TCA-induced effect. Only the 60-week experiment (i.e., Study #1) is useful for the determination of tumor dose-response due to the issues related to appropriateness of control in Study #3. A power calculation of the 60-week study shows that the type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was 41 and 71% for incidence and 7 and 15% for multiplicity of adenomas for the 0.05 and 0.5 g/L TCA exposure groups. For the combination of adenomas and carcinomas, the power was 8 and 92% for incidence and 6 and 56% for multiplicity at 0.05 and 0.5 g/L TCA exposure. Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment.

E.2.3.2.14. ***DeAngelo et al., 1997.*** The design of this study appears to be similar to that of DeAngelo et al. (2008) but to have been conducted in F344 rats. 28−30 day old rats that were reported to be of similar weights were exposed to 2.0 g/L NaCl, 0.05, 0.5, or 5.0 g/L TCA in drinking water for 104 weeks. There were groups of animals sacrificed at 15, 30, 45 and 60 weeks (n = 6) for PCO analysis. There were 23, 24, 19, and 22, animals reported to be examined at terminal sacrifice at 104 weeks and 23, 24, 20, and 22 animals reported to be used in the liver tumor analysis reported by the authors for the control, 0.05, 0.5, and 5.0 g/L treatment groups, respectively. Complete pathological exams were reported to be performed for all tissues from animals in the high dose TCA group at 104 weeks. No indication is given as to whether a complete necropsy and pathological exam was performed for controls at terminal sacrifice. Tritiated thymidine was reported to be administered at interim sacrifices five days prior to sacrifice and to be examined with autoradiography. The 5 g/L TCA treatment group was reported to have a reduction in growth to 89.3% of controls.

For water consumption TCA versus reported to slightly decrease water consumption at all doses with a 7, 8, and 4% decrease in water consumption reported for 0.05, 0.5 and 5.0 g/L TCA, respectively. Body weight was decreased by 5.0 g/L TCA dose only through 78 weeks of exposure to 89.3% of the control value. All of the percent liver/body weight ratios were reported to be slightly decreased (1−4%) by all of the exposure concentrations of TCA but the data shown does not indicate if the liver weight data were taken at interim sacrifice times and appears to be only for animals at terminal sacrifice of 104 weeks.

No data were shown for hepatocyte proliferation but the authors reported no TCA treatment effects. For PCO there was a 2.3-fold difference between control values between the 15-week and 104-week data. For the 0.05 and 0.5 g/L TCA treatment groups there was not a statistically significant difference reported between control and treated group PCO levels. At 15 weeks the PCO activity was reduced by 55%, increased to 1.02-fold, and increased 2.12-fold of control for 0.05, 0.5 and 5.0 g/L TCA exposures, respectively. For the 30 week exposure groups, the 0.05 and 0.5 g/L TCA groups were reported to have PCO levels within 5% of the control level. However, for the 5.0 g/L TCA treatment groups there was ~2-fold of control PCO activity at the 15, 30, 45 and 60 weeks and at 104 weeks there was a 4-fold of control PCO activity. Of note is that the control PCO value was lowest at 104 weeks while the TCA treatment group was similar to interim values.

For analysis of liver tumors, there were 20−24 animals examined in each group. Unlike the study of DeAngelo et al. (2008), it appeared that most of the animals that were sacrificed at 104 weeks were used in the tumor analysis without addition of "extra" animals or deletion of animal data. The incidence of adenomas was reported to be 4.4, 4.2, 15, and 4.6% and the incidence of hepatocellular carcinomas was reported to be 0, 0, 0, and 4.6% for the control, 0.05, 0.5, and 5.0 g/L TCA exposure groups. The multiplicity or tumors/animal was reported to be 0.04, 0.08, 0.15, and 0.05 for adenomas and 0, 0, 0, and 0.05 for carcinomas for the control, 0.05, 0.5, and 5.0 g/L TCA exposure groups. Although there was an increase in the incidence of adenomas at 0.5 g/L and an increase in carcinomas at 5.0 g/L TCA, they were not reported to be statistically significant by the authors. Neither were the increase in adenoma multiplicity at the 0.05 and 0.5 g/L exposures. However, using such a low number of animals per treatment group (n = 20−24) limits the ability of this study to determine a statistically significant increase in tumor response and to be able to determine that there was no treatment-related effect. A power calculation of the study shows that the type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was less than 6% for incidence and multiplicity of tumors at all exposure DCA concentrations with the exception of the incidence of adenomas for 0.5 g/L treatment group (58.7%). Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment. Thus, while suggesting a lower response than for mice for TCA-induced liver tumors, the study is inconclusive for determination of whether TCA induces a carcinogenic response in the liver of rats. The experimental design is such that extrahepatic carcinogenicity of TCA in the male rat cannot be determined.

E.2.3.2.15. *DeAngelo et al., 1996.* In this study, 28-day-old male F344 rats were given drinking water containing DCA at concentrations of 0, 0.05, 0.5, or 5.0 g/L with another group was provided water containing 2.0 g/L NaCl for 100 weeks. This experiment modified its exposure protocol due to toxicity (peripheral neuropathy) such that the 5.0 g/L group was lowered to 2.5 g/L at 9 weeks and then 2.0 g/L at 23 weeks and finally to 1.0 g/L at 52 weeks. When the neuropathy did not reverse or diminish, the animals were sacrificed at 60 weeks and excluded from the results. Based on measured water intake in the 0, 0.05, and 0.5 g/L groups, the time-weighted average doses were reported to be 0, 3.6, and 40.2 mg/kg/d respectively. This experiment was conducted at a U.S. EPA laboratory in Cincinnati and the controls for this group were given 2.0 g/L NaCl (Study #1). In a second study rats were given either deionized water or 2.5 g/L DCA, which was also lowered to 1.5 g/L at 8 weeks and to 1.0 g/L at 26 weeks of exposure (Study #2).

Although 23 animals were reported to be sacrificed at terminal sacrifice that had been given 2 g/L NaCl, the number of animals reported to be examined in this group for hepatocellular lesions was 3. The incidence data for this group for adenomas was 4.4% so this is obviously a typographical error. The number of rats included in the water controls for tumor analysis was reported to be 33 which was the same number as those at final sacrifice. The number of animals at final sacrifice was reported to be 23 for 2 g/L NaCl, 21 for 0.05 g/L DCA, 23 for 0.5 g/L DCA in experiment #1 and 33 for deionized water and 28 for the initial dose of 2.5 g/L DCA in experiment #2. Although these were of the same strain, the initial body weight was 59.1 g versus 76 g for the 2.0 g/L control group versus deionized water group. The treatment groups in both studies were similar to the deionized water group. The percent liver/body weights were greater (4.4 vs. 3.7% in the NaCl vs. deionized water control groups (~20%). The number of unscheduled deaths was greater in Study #2 (22%) than in Study #1 (12%). Interim sacrifice periods were conducted.

As with the DeAngelo et al. (2008) study in mice, the number of animals reported at final sacrifice was not the same as the number examined for liver tumors in Study #1 (5 more animals examined than sacrificed at the 0.05 g/L DCA and 6 more animals examined than sacrificed at the 0.5 g/L DCA exposure groups) with $n = 23$, $n = 26$, and $n = 29$ for the 2 g/L NaCl, 0.05 g/L DCA and 0.5 g/L DCA groups utilized in the tumor analysis. For Study #2 the same number of rats was reported to be sacrificed as examined. The source of the extra animals for tumor analysis in Study #1, whether from interim sacrifice or unscheduled deaths, was not given by the authors and is unknown. Carcinomas prevalence data were not reported for the control group or 0.05 g/L DCA group in Study #1 and multiplicity data were not reported to the control group, or 0.05 g/L DCA group. Multiplicity was not reported for adenomas in the 0.05 g/L DCA group in Study #1.

There was a lack of hepatocyte DNA synthesis and necrosis reported at any dose group carried out to final sacrifice at 100 weeks. The authors reported that the incidence of adenomas to be 4.4% in 2 g/L NaCl control, 0 in 0.05 g/L DCA, and 17.2% in the 0.5 g/L DCA exposure groups. For carcinomas no data were reported for the control or 0.05 g/L DCA group but an incidence of 10.3% was reported for the 0.5 g/L DCA group. The authors reported increased hepatocellular adenomas and carcinomas in male F344 rats although not data were reported for carcinomas in the control and 0.05 g/L exposure groups. They reported that for 0.5 g/L DCA, 24.1 versus 4.4% adenomas and carcinomas combined (Study #1) and 28.6 versus 3.0%

(Study #2) at what was initially 2.5 g/L DCA but continuously reduced). Tumor multiplicity was significantly was reported to be increased in the 0.5 g/L DCA group (0.04 adenomas and carcinomas/animal in control vs. 0.31 in 0.5 g/L DCA in Study #1 and 0.03 in control vs. 0.36 in what was initially 2.5 g/L DCA in Study #2). The issues of use of a small number of animals, additional animals for tumor analysis in Study #1, and most of all the lack of a consistent dose for the 2.5 g/L animals in Study #2, are obvious limitations for establishment of a dose-response for DCA in rats.

E.2.3.2.16. ***Richmond et al., 1995.*** This study was conducted by the same authors as DeAngelo et al. (1996) and appears to report results for the same data set for the 2 g/L NaCl control, 0.05 g/L DCA and 0.5 g/L DCA exposed groups. Of note is that while DeAngelo et al. (1996) refer to the 28-day old rats as "weanlings" the same aged rats are referred to as "adults" in this study. Male Fischer 344 rats were administered time-weighted average concentrations of 0, 0.05, 0.5, or 2.4 g/L DCA in drinking water. Concentrations were kept constant but due to hind-limb paralysis all 2.4 g/L DCA exposed rats had been sacrificed by 60 weeks of exposure. In the 104-week sacrifice time, there were 23 rats reported to be analyzed for incidence of hepatocellular adenomas and carcinomas in the control group, 26 rats in the 0.05 g/L DCA group and 29 rats in the 0.5 g/L DCA exposed group. This is the same number of animals included in the tumor analysis reported in DeAngelo et al. (1996). Tumor multiplicity was not given. Richmond et al. (1995) reported that there was a 4% incidence of adenomas reported in the 2.0 g/L NaCl control animals, 0% at 0.05 g/L DCA, and 21% in the 0.5 DCA group at 104 weeks. These figures are similar to those reported by DeAngelo et al. (1996) for the same data set with the exception of a 17.2% incidence of adenomas reported for the 0.5 g/L DCA group. There were no hepatocellular carcinomas reported in the control or 0.05 g/L exposure groups but a 10% incidence reported in the 0.5 g/L DCA exposure group at 104 weeks of exposure. While carcinomas were not reported by DeAngelo et al. (1996) for the control and 0.05 g/L groups they are assumed to be zero in the summary data for carcinomas and adenomas combined. The 10% incidence at 0.5 g/L DCA is similar to the 10.4% incidence reported for this group by DeAngelo et al. (1996). At 60 weeks at 2.4 g/L DCA, the incidence of hepatocellular adenoma was reported to be 26% and hepatocellular carcinoma to be 4%. This is not similar to the values reported by DeAngelo for 2.5 g/L DCA that was continuously decreased so that the estimated final concentration was 1.6 g/L DCA for 100 weeks for those animals, the incidence of adenomas was reported by DeAngelo et al. (1996) to be 10.7% and carcinomas 21.4%, probably more a reflect of longer exposure time allowing for adenoma to carcinoma progression. The authors did not report any of the results of DCA-induced increases of adenomas and carcinomas to be statistically significant. As it appears the same data set was used for the 2.g/L NaCl control, 0.05 g/L DCA and 0.5 g/L DCA exposure groups as was reported in DeAngelo et al. (1996), the same issues arise as regarding the differences in numbers of animals were included in tumor analysis than were reported to have been present at final sacrifice. As stated previously for the DeAngelo et al. (1997) study of TCA in rats, the use of small numbers of rats limits the detection of and ability to determine whether there was no treatment-related effects, especially at the low concentrations of DCA exposure.

E.2.4. Summaries and Comparisons Between Trichloroethylene (TCE), Dichloroacetic Acid (DCA), and Trichloroacetic Acid (TCA) Studies

There are a number of studies to TCE that have reported effects on the liver. However, the study of this compound is difficult as its concentration does not remain stable in drinking water, some studies have been carried out using TCE with small quantities of a carcinogenic stabilizing agent, some studies have been carried out in whole body inhalation chambers that resulted in additional oral administration and for which individual animal data were not recorded throughout the experiment, and the results of gavage studies have been limited by gavage related deaths and vehicle effects. In addition some studies have been conducted using the i.p. route of administration, which results in route-related toxicity and inflammation. For many studies, liver effects consisted of measured increases in liver weight with little or no description of attendant histological changes induced by TCE treatment. A number of studies were conducted at a few relatively high doses with attendant effects on body weight, indicative of systemic toxicity and affecting TCE-induced liver weight gain. Although, many studies have been performed in male mice, the inhalation studies of Kjellstrand et al. indicate that male mice, regardless of strain appear to have a greater variability in response, as measured by TCE-induced liver weight gain, and susceptibility to TCE-induced decreases in body weight than female mice. However, the body of the TCE literature is consistent in identifying the liver as a target of TCE-induced affects and with the most commonly reported change to be a dose-related TCE-induced increase in liver weight in multiple species, strains, and genders from both inhalation and oral routes of exposure.

The following sections will not only summarize results for studies of TCE reported in Sections E.2.1−E.2.2, but provide comparison of studies of either TCA or DCA that have used similar paradigms or investigated similar parameters described in Sections E.2.3.1 and E.2.3.2. A synopsis of the results from studies of CH and in comparison with TCE results is presented in Section E.2.5. While the study of Bull et al. (2002), described in Section E.2.2.21, presents data for combinations of DCA or TCA exposure for comparisons of tumor phenotype with those induced by TCE, the examination of coexposure studies of TCE metabolites in rodents that are also exposed to a number of other carcinogens, and descriptions of the toxicity data for brominated haloacetates that also occur with TCE in the environment, are presented in Section E.4.3.3.

E.2.4.1. *Summary of Results For Short-term Effects of Trichloroethylene (TCE)*

In regard to early changes in DNA synthesis, the data for TCE is very limited. The study by Mirsalis et al. (1989) used an *in vivo-in vitro* hepatocyte DNA repair and S-phase DNA synthesis in primary hepatocytes from male Fischer-344 rats (180−300 g) and male and female B6C3F1 mice (20−29 g for male mice and 18−25 g female mice) administered TCE by gavage in corn oil. They reported negative results 2−12 hours after treatment from 50−1,000 mg/kg TCE in rats and mice (male and female) for unscheduled DNA synthesis and repair using 3 animals per group. After 24 and 48 hours of 200 or 1,000 mg/kg TCE in male mice (n = 3) and after 48 hours of 200 (n = 3) or 1,000 (n = 4) mg/kg TCE in female mice, similar values of 0.30 to 0.69% of hepatocytes were reported as undergoing DNA synthesis in those hepatocytes in primary culture with only the 1,000 mg/kg TCE dose in male mice at 48 hours giving a result considered to be positive (~2.2%). No statistical analyses were performed on these measurements, which were obviously limited by both the number of animals examined and the relevance of the paradigm.

TCE-induced increases in liver weight have been reported to occur quickly. The inhalation study of Okino et al. (1991) in male rats demonstrates that liver weight and metabolism were increased with as little as 8 hours of TCE exposure (500 and 2,000 ppm) and as early as 22 hours after cessation of such exposures with little concurrent hepatic necrosis. Laughter reported increase liver weight in SV129 mice in their 3-days study (see below). Tao et al. (2000) reported a 1.26-fold of control percent liver/body weight in female B6C3F1mice fed 1,000 mg/kg TCE in corn oil for 5 days. Elcombe et al. (1985) and Dees and Travis (1993) reported gavage results in mice and rats after 10 days exposure to TCE which showed TCE-induced increases in liver weight (see below for more detail on dose-response). Tucker et al. (1982) reported that 14 days of exposure to 24 mg/kg and 240 mg/kg TCE via gavage to induce a dose-related increase in liver weight in male CD-1 mice but did not show the data.

TCE-induced increases in percent liver/body weight ratios have been studied most extensively in B6C3F1 and Swiss mice. Both strains have been shown to have a TCE-induced increase in liver tumors from long-term exposure as well (see Section E.2.4.2, below). A number of studies have provided dose-response information for TCE-induced increases in liver weight from 10 days to 13 weeks of exposure in mice. Most studies have reported that the magnitude of increase in TCE exposure concentration is similar to the magnitude increase of percent liver/body weight increase. For example a 2-fold increase in TCE exposure has often resulted in a 2-fold increase in the percent change in liver/body weight over control (i.e., 500 mg/kg TCE induces a

20% increase in liver weight and 1,000 mg/kg TCE induces a 50% increase in liver weight as reported by Elcombe et al., 1985). The range in which this relationship is valid has been reported to vary from 100 mg/kg TCE at 10 days (Dees and Travis, 1993) to 1,600 mg/kg (Buben and O'Flaherty, 1985) at 6 weeks and up to 1,500 mg/kg TCE for 13 weeks (NTP, 1990). The consistency in the relationship between magnitude of liver weight increase and TCE exposure concentration has been reported for both genders of mice, across oral and inhalation routes of exposure, and across differing strains of mice tested. For rats, there are fewer studies with fewer exposure levels tested, but both Berman et al. (1995) and Melnick et al. (1987) report that short-term TCE exposures from 150 mg/kg to ~2,000 mg/kg induced percent liver/body weight that increased proportionally with the magnitude of TCE exposure concentration.

Dependence of PPARα activation for TCE-liver weight gain has been investigated in PPARα null mice by both Nakajima et al. (2000) and Laughter et al. (2004). After 2 weeks of 750 mg/kg TCE exposure to carefully matched SV129 wild-type or PPARα-null male and female mice (n = 6 group), there was a reported 1.50-fold of control in wild-type and 1.26-fold of control percent liver/body weight in PPARα-null male mice by Nakajima et al. (2000). For female mice, there was ~1.25-fold of control percent liver/body weight ratios for both wild-type and PPARα-null mice. Thus, TCE-induced liver weight gain was not dependent on a functional PPARα receptor in female mice and some portion of it may have been in male mice. Both wild-type male and female mice were reported to have similar increases in the number of peroxisome in the pericentral area of the liver and TCE exposure and, although increased 2-fold, were still only ~4% of cytoplasmic volume. Female wild-type mice were reported to have less TCE-induced elevation of very long chain acyl-CoA synthetase, D-type peroxisomal bifunctional protein, mitochondrial trifunctional protein α subunits α and β, and cytochrome P450 4A1 than males mice, even though peroxisomal volume was similarly elevated in male and female mice. The induction of PPARα protein by TCE treatment was also reported to be slightly less in female than male wild-type mice (2.17- vs. 1.44-fold of control, respectively).

Laughter et al. (2004) also studied SV129 wild-type and PPARα-null male mice treated with 3 daily doses of TCE in 0.1% methyl cellulose for either 3 days (1,500 mg/kg TCE) or 3 weeks (0, 10, 50, 125, 500, 1,000, or 1,500 mg/kg TCE 5 days a week). However, not only is the paradigm not comparable to other gavage paradigms, but no initial or final body weights of the mice were reported and thus, the influence of differences in initial body weight on percent liver/body weight determinations could not be ascertained. In the 3-day study, while control wild-type and PPARα-null mice were reported to have similar percent liver/body weight ratios (~4.5%), at the end of the 3-week experiment the percent liver/body weight ratios were reported to be increased in the PPARα-null male mice (5.1%). TCE treatment for 3 days was reported to increase the percent liver/body weight ratio 1.4-fold of control in the wild-type mice and 1.07-fold of control in the null mice. In the 3-week study, wild-type mice exposed to various concentrations of TCE had percent liver/body weights that were reported to be within ~2% of control values except for the 1,000 mg/kg and 1,500 mg/kg groups (~1.18- and 1.30-fold of control levels, respectively). For the PPARα-null mice the variability in percent liver/body weight was reported to be greater than that of the wild-type mice in most of the groups and the baseline level of percent liver/body weight ratio also 1.16-fold greater. TCE exposure was apparently more toxic in the null mice with death at the 1,500 mg/kg TCE exposure level resulting in the prevention of recording of percent liver/body weights. At 1,000 mg/kg TCE exposure level there was a reported 1.10-fold of control percent liver/body weight in the PPARα-null mice. None of the increases in percent liver/body weight in the null mice were reported to be statistically significant by Laughter et al. (2004). However, the statistical power of the study was limited due to low numbers of animals and increased variability in the null mice groups. The percent liver/body weight after TCE treatment that was reported in this study was actually greater in the null mice than the wild-type male mice at the 1,000 mg/kg TCE exposure level (5.6% ± 0.4% vs. 5.2% ± 0.5%, for null and wild-type mice, respectively). At 1-weeks and at 3-weeks, TCE appeared to induce increases in liver weight in PPARα-null mice, although not reaching statistical significance in this study. At a 1,000 mg/kg TCE exposure for 3 weeks percent liver/body weights were reported to be 1.18-fold of control in wild-type and 1.10-fold of control in null mice. Although the experiments in Laughter et al. for DCA and TCA were not conducted using the same paradigm, the TCE-induced increase in percent liver/body weight more closely resembled the dose-response pattern for DCA than for DCA wild-type SV129 and PPARα-null mice.

Many studies have used cyanide-insensitive PCO as a surrogate for peroxisome proliferation. Of note is that several studies have shown that this activity is not correlated with the volume or number of peroxisomes that are increased as a result of exposure to TCE or it metabolites (Nakajima et al.,2000; Elcombe et al., 1985: Nelson et al., 1989). This activity appears to be highly variable both as a baseline measure and in response to chemical exposures. Laughter et al. (2004) presented data showing that WY-14,643 induced increases in PCO activity varied up to 6-fold between experiments in wild-type mice. They also showed that PCO activity, in some instances, was up to 6-fold of wild-type mice values in untreated PPARα-null mice. Parrish et al. (1996) noted that control values between experiments varied as much as a factor of 2-fold for PCO activity and thus, their data were presented as percent of concurrent controls. Goldsworthy and Popp (1987) reported that 1,000 mg/kg TCE induced a 6.25-fold of control PCO activity in B6C3F1 mice in two 10-day experiments. However, for F344 rats, the increases over control between two experiments conducted at the same dose were reported to vary by >30%. Finally, Melnick et al. (1987) have reported that corn oil administration alone can elevate PCO activity as well as catalase activity.

For TCE there are two key 10-days studies (Elcombe et al., 1985; Dees and Travis, 1993) that examine the effects of short-term exposure in mice and rats via gavage exposure and attempt to determine the nature of the dose response in a range of exposure concentrations that include levels below which there is concurrent decreased body weights. Although they have limitations, they reported generally consistent results. In regard to liver weight in mice, gavage exposure to TCE at concentrations ranging from 100 to 1,500 mg/kg TCE produced increases in liver/body weight that was dose-related (Elcombe et al., 1985; Dees and Travis, 1993).

Elcombe et al. (1985) reported a small decrease in DNA content with TCE treatment (consistent with hepatocellular hypertrophy) that was not dose-related, increased tritiated thymidine incorporation in whole mouse liver DNA that was that was treatment but not dose-related (i.e., a 2-, 2-, and 5-fold of control values in mice treated with 500, 1,000, and 1,500 mg/kg TCE), and slightly increased numbers of mitotic figures that were treatment but not dose-related and not correlated with DNA synthesis as measured by thymidine incorporation. Elcombe et al. (1985) reported an increase in peroxisome volume after TCE exposure that was correlated with the magnitude of increase in peroxisomal-associated enzyme activity at the only dose in which both were tested. Peroxisome increases after TCE treatment in mice livers were identified as being pericentral in location. After TCE treatment, increased peroxisomal volumes in B6C3F1 mice were reported to be not dose-related (i.e., there was little difference between 500 to 1,500 mg/kg TCE exposures). The TCE-induced increases in peroxisomal volumes were also not correlated with the reported increases in thymidine incorporation or mitotic activity in mice. Neither TCE-induction of peroxisomes or hepatocellular proliferation, as measured by either mitotic index or thymidine incorporation, was correlated with TCE-induced liver weight increases. Elcombe et al. (1985) only measured PCO activity in a subset of B6C3F1 mice at the 1,000 mg/kg TCE exposure level for 10 days of exposure and reported an 8-fold of control PCO activity and a 1.5-fold of control catalase activity. This result was similar to that of Goldsworthy and Popp (1987) who reported 6.25-fold of control PCO activity in male B6C3F1 mice exposed to 1,000 mg/kg/d TCE for 10 days in two separate experiments.

Similar to Elcombe et al., who reported no difference in response between 500 and 1,000 mg/kg TCE treatments, Dees and Travis (1993) reported that incorporation of tritiated thymidine in DNA from mouse liver was elevated after TCE treatment and the mean peak level of tritiated thymidine incorporation occurred at 250 mg/kg TCE treatment level remaining constant for the 500 and 1,000 mg/kg treated groups. Dees and Travis (1993) specifically report that mitotic figures, although very rare, were more frequently observed after TCE treatment, found most often in the intermediate zone, and found in cells resembling mature hepatocytes. They reported that there was little tritiated thymidine incorporation in areas near the bile duct epithelia or close to the portal triad in liver sections from both male and female mice. They also reported no evidence of increased lipofuscin and that increased apoptoses from TCE exposure “did not appear to be in proportion to the applied TCE dose given to male or female mice” (i.e., the mean number of apoptosis 0, 0, 0, 1 and 8 for control, 100, 250, 500, and 1,000 mg/kg TCE treated groups, respectively). Both Elcombe et al. (1985) and Dees and Travis (1993) reported no changes in apoptosis other than increased apoptosis only at a treatment level of 1,000 mg/kg TCE.

Elcombe et al. (1985) reported increased in percent liver/body weight after TCE treatment in both the Osborne-Mendel and Alderly Park rat strain, although to a smaller extent than in mice. For both strains, Elcombe et al. (1985) reported no TCE-induced changes in body weight at doses ranging from 500 to 1,500 mg/kg. For male Osborne-Mendel rats administration of TCE in corn oil gavage resulted in a 1.18-, 1.26-, and 1.30-fold of control percent liver/body weight at 500 mg/kd/day, 1,000 mg/kg/d, and 1,500 mg/kg/d exposures, respectively. For Alderly Park rats those increases were 1.14-, 1.17-, and 1.17-fold of control at the same respective exposure levels for 10 days of exposure. In regard to liver weight increases, Melnick et al. (1987) reported a 1.13- and 1.23-fold of control percent liver/body weight in male Fischer 344 rats fed 600 mg/kg/d and 1,300 mg/kg/d TCE in capsules, respectively. There was no difference in the extent of TCE-induced liver increase between the two lowest dosed group administered TCE in corn oil gavage (~20% increase in percent liver/body weight at 600 mg/kd and 1,300 mg/kg TCE) for 14 days. However, the magnitude of increases in percent liver/body weight in these groups was affected by difference between control groups in liver weight although initial and final body weights appeared to be similar. By either type of vehicle, Melnick et al. (1987) reported decreases in body weights in rats treated with concentrations of TCE 2,200 mg/kg/d or greater for 14 days. Similarly, Nunes et al. (2001) reported decreased body weight in S-D rats administered 2,000 mg/kg/d for 7 days in corn oil. Melnick et al. (1987) reported that both exposures to either 600 or 1,300 mg/kg/d TCE in capsules did not result in decreased body weight and caused less than minimal focal necrosis randomly distributed in the liver. At 2,200 and 4,800 mg/kg TCE fed via capsule, Melnick et al. (1987) reported that although there was decreased body weight in rats treated at these exposures, there was little TCE-induced necrosis, and no evidence of inflammation, cellular hypertrophy or edema with TCE exposure. Similarly, Berman et al. (1995) reported increases in liver weight gain at doses as low as 50 mg/kg TCE, no necrosis up to doses of 1,500 mg/kg, and hepatocellular hyper trophy only at the 1,500 mg/kg level in female Fischer 344 rats.

For rats, Elcombe et al. (1985) reported an increase over untreated rats of 1.13-fold of control PCO activity in Alderly Park rats after 1,000 mg/kg/d TCE exposure for 10 days, while Goldsworthy and Popp (1987) reported a 1.8- and 2.39-fold of control in male Fischer 344 rats at the same exposure in two separate experiments. Melnick et al. (1987) reported PCO activity of 1.23- and 1.75-fold of control in male Fischer 344 rats fed 600 mg/kg/d and 1,300 mg/kg/d TCE for 14 days in capsules. For rats treated by gavage with 600 mg/kg/d or 1,200 mg/kg d TCE corn oil, they reported 1.16- and 1.29-fold of control values. However, control levels of PCO were 16% higher in corn oil controls than in untreated controls. In addition Melnick et al. (1987) reported little catalase increases in rats fed TCE via capsules in food (less than 6% increase) but a 1.18- and 1.49-fold of control catalase activity in rats fed 600 mg/kg/d or 1,200 mg/kg/TCE via corn oil gavage, indicative of a vehicle effect.

The data from Elcombe et al. (1985) included reports of TCE-induced pericentral hypertrophy and eosinophilia for both rats and mice but with "fewer animals affected at lower doses." In terms of glycogen deposition, Elcombe report "somewhat" less glycogen pericentrally in the livers of rats treated with TCE at 1,500 mg/kg than controls with less marked changes at lower doses restricted to fewer animals. They do not comment on changes in glycogen in mice. Dees and Travis (1993) reported TCE-induced changes to "include an increase in eosinophilic cytoplasmic staining of hepatocytes located near central veins, accompanied by loss of cytoplasmic vacuolization." Since glycogen is removed using conventional tissue processing and staining techniques, an increase in glycogen deposition would be expected to increase vacuolization and thus, the report from Dees and Travis is consistent with less not more glycogen deposition. Neither study produced a quantitative analysis of glycogen deposition changes from TCE exposure. Although not explicitly discussing liver glycogen content or examining it quantitatively in mice, these studies suggest that TCE-induced liver weight increases did not appear to be due to glycogen deposition after 10 days of exposure and any decreases in glycogen were not necessarily correlated with the magnitude of liver weight gain either.

For both rats and mice the data from Elcombe et al. (1985) showed that tritiated thymidine incorporation in total liver DNA observed after TCE exposure did not correlate with mitotic index activity in hepatocytes with both Elcombe et al. (1985) and Dees and Travis (1993) reporting a small mitotic indexes and evidence of periportal hepatocellular hypertrophy from TCE exposure. Neither mitotic index or tritiated thymidine incorporation data support a correlation with TCE-induced liver weight increase in the mouse. If higher levels of hepatocyte replication had occurred earlier, such levels were not sustained by 10 days of TCE exposure. Both Elcombe et al. (1985) and Dees and Travis (1993) present data that represent "a snapshot in time" which does not show whether increased cell proliferation may have happened at an earlier time point and then subsided by 10 days. These data suggest that increased tritiated thymidine levels were targeted to mature hepatocytes and in areas of the liver where greater levels of polyploidization occur. Both Elcombe et al. (1985) and Dees and Travis (1993) show that tritiated thymidine incorporation in the liver was ~2-fold of controls between 250−1,000 mg/kg TCE, a result consistent with a doubling of DNA. Thus, given the normally quiescent state of the liver, the magnitude of this increase over control levels, even if a result of proliferation rather than polyploidization, would be confined to a very small population of cells in the liver after 10 days of TCE exposure. Laughter et al. (2004) reported that there was an increase in DNA synthesis after aqueous gavage exposure to 500 and 1,000 mg/kg TCE given as 3 boluses a day for 3 weeks with BrdU given for the last week of treatment. An examination of DNA synthesis in individual hepatocytes was reported to show that 1 and 4.5% of hepatocytes had undergone DNA synthesis in the last week of treatment for the 500 and 1,000 mg/kg doses, respectively. Both Elcombe et al. (1985) and Dees and Travis (1993) show TCE-induced changes for several parameters at the lowest level tested without toxicity and without evidence of regenerative hyperplasia or sustained hepatocellular proliferation. In regards to susceptibility to liver cancer induction, the more susceptible (B6C3F1) versus less susceptible (Alderly Park/Swiss) strains of mice to TCE-induced liver tumors (Maltoni et al., 1988), the "less susceptible" strain was reported by Elcombe et al. (1985) to have, a greater baseline level of liver weight/body weight ratio, a greater baseline level of thymidine incorporation as well as greater responses for those endpoints due to TCE exposure. However, both strains showed a hepatocarcinogenic response after TCE exposure, although there are limitations regarding determination of the exact magnitude of response for these experiments as previously discussed.

E.2.4.2. *Summary of Results For Short-Term Effects of Dichloroacetic Acid (DCA) and Trichloroacetic Acid (TCA): Comparisons With Trichloroethylene (TCE)*

Short-term exposures from DCA and TCA have been studied either through gavage or in drinking water. Palatability became an issue at the highest level of DCA tested in drinking water experiments (5 g/L) which caused a significant reduction of drinking water intake in mice of 46 to 64% (Carter et al., 1995). Decreases in drinking water consumption have also been reported for a range of concentrations of DCA and TCA from 0.05 g/L to 5.0 g/L, in both mice and rats, and with generally the higher concentrations producing the highest decrease in drinking water (Carter et al., 1995; Mather et al., 1990; DeAngelo et al., 1997, 1999, 2008). However, results within studies (e.g., DeAngelo et al., 2008) and between studies have been reported to vary as to the extent of the reduction in drinking water from the presence of TCA or DCA. Some drinking water studies of DCA or TCA have not reported drinking water consumption as well. Therefore, although in general DCA and TCA studies have do not include vehicle effects, such as corn oil, they have been affected by differences in drinking water consumption not only changing the dose received by the rodents and therefore, potentially the shape of the dose-response curve, but also the effects of dehydration are potentially added to any chemically-related reported effects.

Studies have attempted to determine short-term effects on DNA by TCE and its metabolites. Nelson and Bull (1988) administered TCE male Sprague Dawley rats and male B6C3F1 mice measured the rate of DNA unwinding under alkaline conditions 4 hours later. For rats there was a significantly increased rate of unwinding at the two highest dose and for mice there was a significantly increased level of DNA unwinding at a lower dose. In this same study, DCA was reported to be most potent in this assay with TCA being the lowest, while CH closely approximated the dose-response curve of TCE in the rat. In the mouse the most potent metabolite in the assay was reported to be TCA followed by DCA with CH considerably less potent. Nelson and Bull (1988) and Nelson et al. (1989) have reported increases in single strand breaks after DCA and TCA exposure. However, Styles et al. (1991) (for mice) and Chang et al. (1992) (for mice and rats) did not. Austin et al. (1996) note that the alkaline unwinding assay, a variant of the alkaline elution procedure, is noted for its variability and inconsistency depending on the techniques used while performing the procedure. In regard to oxidative damage as measured by TBARS for lipid peroxidation and 8-OHdG levels in DNA, increases appear to be small (less than 50% greater than control levels) and transient after DCA and TCA treatment in mice (see Section E.3.4.2.3) with TCE results confounded by vehicle or route of administration effects.

Although there is no comparative data for TCE, the study of Styles et al. (1991) is particularly useful for determining effects of TCA from 1 to 4 days of exposure in mice. Styles et al. (1991) reported no change in “hepatic” DNA uptake of tritiated thymidine up to 36 hours, a peak at 72 hours (~6-fold of control), and falling levels by 96 hours (~4-fold of controls) after 500 mg/kg TCA gavage exposure. Incorporation of tritiated thymidine observed for individual hepatocytes decreased between 24 and 36 hours, rose slowly back to control levels at 48 hours, significantly increased by 72 hours, and then decreased by 96 hours. Thus, increases in “hepatic” DNA tritiated thymidine uptake did not capture the decrease observed in individual hepatocytes at 36 hours. By either measure the population of cells undergoing DNA synthesis was small with the peak level being less than 1% of the hepatocyte population. Zonal distribution of labeled hepatocytes were decreased at 36 hours in all zones, appeared to be slightly greater in perioportal than midzonal cells with centrilobular cells still below control levels by 48 hours, similarly elevated over controls in all zones by 72 hours, and to have returned to near control levels in the midzonal and centrilobular regions but with periportal areas still elevated by 96 hours. These results are consistent with all hepatocytes showing a decrease in DNA synthesis by 36 hours and then a wave of DNA synthesis to occur, starting at the periportal zone and progressing through the liver acinus that is decreased by 4 days after exposure.

Along with changes in liver weight, DNA synthesis, and glycogen accumulation, several studies of DCA and TCA have focused on the extent of peroxisome proliferation as measured by changes in peroxisome number, cytoplasmic volume and enzyme activity induction as potential "key events" occurring from shorter-term exposures that may be linked to chronic effects such as liver tumorigenicity. As noted above in Section E.2.4.1, TCE-induced liver weight gain has been reported to not be dependent on a functional PPARα receptor in female mice while some portion of increased liver weight may have been in male mice. Also as noted cyanide-insensitive PCO has also been reported to not be correlated with the volume or number of peroxisomes that are increased as a result of exposure to TCE or it metabolites (Nakajima et al., 2000; Elcombe et al., 1985: Nelson et al., 1989) and to be highly variable both as a baseline measure and in response to chemical exposures (e.g., variation of up to 6-fold between after WY-14,643 exposure in mice). Also as noted, above the vehicle used in many TCE gavage experiments, corn oil, has been reported to elevate PCO activity as well as catalase activity.

A number of short-term studies have examined the effects of TCA and DCA on liver weight increases and evidence of peroxisome proliferation and changes in DNA synthesis. In particular two studies of DCA and TCA used a similar paradigm presented by Elcombe et al. (1985) and Dees and Travis (1993) for TCE effects in mice. Nelson et al. (1989) report findings from gavage doses of unbuffered TCA (500 mg/kg) and DCA (500 mg/kg) in male B6C3F1 mice and Styles et al. (1991) also providing data on peroxisome proliferation using the same paradigm. Nelson et al. (1989) reported levels of PCO activity in mice administered 500 mg/kg DCA or TCA for 10 days with 250 mg/kg Clofibrate administration serving as a positive control. DCA and TCA exposure were reported to not affect body weight, but both to significantly increase liver weight (1.63-fold of control for DCA and 1.30-fold of control for TCA treatments), and percent liver/body weight ratios (1.53-fold of control for DCA and 1.16-fold of control for DCA treatments). PCO activity was reported to be significantly increased by ~1.63-, 2.7-, and 5-fold of control for DCA, TCA and Clofibrate treatments, respectively and indicated that both DCA and TCA were weaker inducers of this activity than Clofibrate. Results from randomly selected electron photomicrographs showed an increase in peroxisomes per unit area but gave a different pattern than PCO enzyme activity (i.e., 2.5- and 2.4-fold of control peroxisome volume for DCA and TCA, respectively). Evidence of gross hepatotoxicity was reported to not occur in vehicle or TCA-treated mice. Light microscopic sections were reported to show TCA and control hepatocytes to have the same intensity of PAS staining, but with slightly larger hepatocytes occurring in TCA-treated mice throughout the liver section with architecture and tissue pattern of the liver intact. For DCA, the histopathology was reported to be markedly different than control mice or TCA treated mice. DCA was reported to induce a marked increase in the size of hepatocytes throughout the liver with an approximately 1.4-fold of control diameter that was accompanied by increased PAS staining (indicative of glycogen deposition). All DCA-treated mice were reported to have multiple white streaks grossly visible on the surface of the liver corresponding with subcapsular foci of coagulative necrosis that were not encapsulated, varied in size, and accompanied by a slight inflammatory response characterized by neutrophil infiltration.

A quantitative comparison of effects from equivalent exposures of TCE, TCA, and DCA (500 mg/kg for 10 days in mice via corn oil gavage for TCE) shown in Table E-13 can be drawn between the Elcombe et al. (1985), Dees and Travis (1993), Styles et al. (1991), and Nelson et al. (1989) data for relationship to control values for percent liver/body weight, PCO, and qualitatively for glycogen deposition.

Table E-13. Comparison of liver effects from TCE, TCA, and DCA (10-day exposures in mice)

Model	Expo-sure	% Liver/body wt.	Peroxisome volume	Peroxisome enzyme activity	Glycogen deposition
Nelson et al., 1989[a]					
B6C3F1 male	TCA	1.16-fold	2.4-fold	2.7-fold	No change
	DCA	1.53-fold	2.5-fold	1.63-fold	Increased
Styles et al., 1991					
B6C3F1 male	TCA	NR	1.9-fold	NR	NR
Elcombe et al., 1985					
B6C3F1 male	TCE	1.20-fold	8-fold	NR	NR
Alderly Park male (Swiss)	TCE	1.43-fold	4-fold	NR	NR
Dees and Travis, 1993					
B6C3F1 male	TCE	1.05-fold[b]	NR	NR	NR
B6C3F1 female	TCE	1.18-fold	NR	NR	NR

[a]Unbuffered. NR = not reported as no analysis was performed for this dose or the authors did not report this finding (i.e., did not note a change in glycogen in description of exposure-related changes).
[b]Statistically significant although small increase.

Although using a similar species, route of exposure, and dose, the comparison of responses for TCE and its metabolites shown above are in male mice and also are reflective of variability in strain, and variability and uncertainty of initial body weights. As described in more detail in Section E.2.2, initial age and body weight have an impact on TCE-related increases in liver weight. Male mice have been reported to have greater variability in response than female mice within and between studies and most of the comparative data for the 10-day 500 mg/kg doses of TCE or its metabolites were from studies in male mice. Corn oil, used as the vehicle for TCE gavage studies but not those of its metabolites, has been noted to specifically affect peroxisomal enzyme induction, body weight gain, and hepatic necrosis, specifically, in male mice (Merrick et al., 1989). Corn oil alone has also been reported to increase PCO activity in F344 rats and to potentiate the induction of PCO activity of TCA (DeAngelo et al., 1989). Thus, quantitative inferences regarding the magnitude of response in these studies are limited by a number of factors.

The variability in the magnitude of TCE-induced increases in percent liver/body weight across studies in readily apparent but for TCE, TCA and DCA there is an increase in liver weight in mice at this dose after 10 days of exposure. The volume of the peroxisomal compartment in hepatocytes was reported to be more greatly increased from TCE-treatment by Elcombe et al. (1985) than for either TCA or DCA by Nelson et al. (1989) or Styles et al. (1991). However, the control values for the B6C3F1 mice were half that of the other strain reported by Elcombe et al. (1985) and this parameter in general did not match the pattern of PCO activity values reported for TCA and DCA (Nelson et al., 1989). There is no PCO activity data at this dose for TCE but Elcombe et al. (1985) reported that the magnitude of TCE-induced increase in peroxisome volume was similar to that of PCO activity at the only dose where both were tested (1,000 mg/kg TCE). However, Elcombe et al. (1985) reported increased peroxisomal volumes in B6C3F1 mice after 10 days of TCE treatment were not dose-related (i.e., there was little difference between 500, 1,000, and 1,500 mg/kg TCE exposures in the magnitude of TCE-induced increases in peroxisomal volume). The lack of dose-response for TCE-induced peroxisomal volume increases was not consistent with increases in percent liver/body weight that increased with increasing TCE exposure concentration. Also as noted above, PCO activity appears to be highly variable in untreated and treated rodents and to vary between experiments and between studies.

From the above comparison it is clears that TCE, DCA and TCA exposures were associated with increased liver weight in mice but a question arises as to what changes account for the liver weight increases. For TCE and TCA 500 mg/kg treatments, changes in glycogen were not reported in the general descriptions of histopathological changes (Elcombe et al., 1985; Styles et al., 1991; Dees and Travis, 1993) or were specifically described by the authors as being similar to controls (Nelson et al., 1989). However, for DCA, glycogen deposition was specifically noted to be increased with treatment, although no quantitative analyses was presented that could give information as to the nature of the dose-response (Nelson et al., 1989). Issues in regard to not only whether TCE and its metabolites each gives a similar response for a number of parameters, but what potential changes may be associated with carcinogenicity from long-term exposures can be examined by a comparison of the dose-response curves for these parameters from a range of exposure concentrations and durations of exposure. In addition, if glycogen accumulation results from DCA exposure, what proportion of DCA-induced liver weight increases result from such accumulation or other events that may be similar to those occurring with TCE exposure (see Section E.4.2.4, below)?

As noted above in Section E.2.4.1., TCE-induced changes in liver weight appear to be proportional to the exposure concentration across route of administration, gender and rodent species. As an indication of the potential contribution of TCE metabolites to this effect, a comparison of the shape of the dose-response curves for liver weight induction for TCE and its metabolites is informative. A number of studies of TCA and DCA in drinking water, conducted from 10-days to 4 weeks, have attempted to measure changes in liver weight induction, peroxisomal enzyme activity, and changes in DNA synthesis predominantly in mice to provide insight into the MOA(s) for liver cancer induction (Parrish et al., 1996; Sanchez and Bull, 1990; Carter et al., 1995; DeAngelo et al., 1989, 2008).

Direct comparisons are harder to make between the drinking water studies of DCA and TCA and the gavage studies of TCE (Tables E-14, E-15, and E-16). Similar to 10-day gavage exposures to TCE, 14-day exposures to TCA or DCA via drinking water were reported to induce dose-related increases in liver weight in male B6C3F1 mice (0.3, 1.0, and 2.0 g/L TCA or DCA) with a greater increase in liver weight from DCA than TCA at 2 g/L and a difference in the shape of the dose-response curve (Sanchez and Bull, 1990). They reported a 1.08-, 1.31-, and 1.62-fold of control liver weight for DCA and a 1.15-, 1.22-, and 1.38-fold of control values for TCA at 0.3 g/L, 1.0 g/L and 2.0 g/L concentrations, respectively (n = 12−14 mice). While the magnitude of difference between the exposures was ~6.7-fold between the lowest and highest dose, the differences between TCA exposure groups for change in percent of liver weight was ~2.5, but for DCA the slope of the dose-response curve for liver weight increases appeared to be closer to the magnitude of difference in exposure concentrations between the groups (i.e., a difference of 7.7-fold between the highest and lowest dose for liver weight induction).

Table E-14. Liver weight induction as percent liver/body weight fold-of-control in male B6C3F1 mice from DCA or TCA drinking water studies

Concentration (g/L)	Duration of exposure				Mean for average of days 14−30
	14 or 15 days	20 or 21 days	25 days	28 or 30 days	
DCA					
0.1		1.02-fold			1.02-fold
0.3	1.08-fold				1.08-fold
0.5	1.12-fold	1.24-fold, 1.05-fold	1.16-fold	1.16-fold	1.15-fold
1.0	1.31-fold				1.31-fold
2.0	1.62-fold	1.46-fold, 2.01-fold	2.04-fold	1.99-fold, 1.42-fold	1.83-fold
5.0	1.67-fold				1.67-fold
TCA					
0.05				1.09-fold	1.09-fold
0.1		0.98-fold			0.98-fold
0.3	1.15-fold				1.15-fold
0.5		1.13-fold		1.16-fold	1.15-fold
1.0	1.23-fold, 1.08-fold				1.16-fold
2.0	1.38-fold, 1.16-fold, 1.26-fold	1.33-fold			1.30-fold
3.0				1.33-fold	1.33-fold
5.0	1.39-fold, 1.35-fold				1.37-fold

Table E-15. Liver weight induction as percent liver/body weight fold-of-control in male B6C3F1 or Swiss mice from TCE gavage studies

Concentration (mg/kg/d)	10 days	28 days	42 days	Mean for average of days 10−42
B6C3F1				
100	1.00-fold			1.00-fold
250	1.00-fold			1.00-fold
500	1.20-fold, 1.06-fold			1.13-fold
600		1.36-fold		1.36-fold
1,000	1.50-fold, 1.17-fold, 1.50-fold			1.39-fold
1,200		1.64-fold		1.64-fold
1,500	1.47-fold			1.47-fold
2,400		1.81-fold		1.81-fold
Swiss				
100			1.12-fold	1.12-fold
200			1.15-fold	1.15-fold
400			1.25-fold	1.25-fold
500	1.43-fold	1.32-fold		1.38-fold
800			1.36-fold	1.36-fold
1,000	1.56-fold	1.41-fold		1.49-fold
1,500	1.75-fold			1.75-fold
1,600			1.63-fold	1.63-fold
2,000		1.38-fold		1.38-fold
2,400		1.69-fold		1.69-fold

Table E-16. B6C3F1 and Swiss (data sets combined)

Concentration (mg/kg/d)	Mean for average of days 10−42
100	1.06-fold
200	1.15-fold
250	1.00-fold
400	1.25-fold
500	1.26-fold
600	1.36-fold
800	1.36-fold
1,000	1.49-fold
1,200	1.64-fold
1,500	1.61-fold
1,600	1.63-fold
2,000	1.38-fold
2,400	1.75-fold

DeAngelo et al. (1989) reported that after 14 days of exposure to 5 g/L or 2 g/L TCA in male mice, the magnitudes of the difference in the increase in dose (2.5-fold) was generally higher than the increase percent liver/body weight ratios at these doses (i.e., ~40% for the Swiss-Webster, C3H, and for one of the B6C3F1 mouse experiments, and for the C57BL/6 mouse there was no difference in liver weight induction between the 2 and 5 g/L TCA exposure groups). There was a range in the magnitude of percent liver/body weight ratio increases between the strains of mice with liver weight induction reported to range between 1.26- to 1.66-fold of control values for the 4 strains of mice at 5 g/L TCA and to range between 1.16- to 1.63-fold of control values at 2 g/L TCA. One strain, B6C3F1, was chosen to compare responses between DCA and TCA. At 1 g/L, 2 g/L and 5 g/L TCA or DCA, DCA was reported to induce a greater increase in liver weight that TCA (i.e., 1.55- vs. 1.39-fold of control percent liver/body weight ratio for 5.0 g/L DCA vs. TCA, respectively). At the 5 g/L exposures DCA induced ~40% greater percent liver/body weight than TCA. Although as noted above, the majority of the data from this study in mice did not indicate that the magnitude of difference in exposure concentration was the same as that of liver weight induction for TCA, in the particular experiment that examined both DCA and TCA, the increase in percent liver/body weight ratios were similar to the magnitude of difference in dose between the 2 g/L and 5 g/L exposure concentrations for both DCA and TCA (i.e., 2- to

2.5-fold increase in liver weight change corresponding to a 2.5-fold difference in exposure concentration).

Carter et al. (1995) examined 0.5 and 5.0 g/L exposures to DCA in B6C3F1 male mice and reported that percent liver/body weights were increased consistently from 0.5 g/L DCA treatment from 5 days to 30 days of treatment (i.e., a range of 1.05- to 1.16-fold of control). For 5.0 g/L DCA exposure the range of increase in percent liver/body weight was reported to be 1.37- to 2.04-fold of control for the same time period. At the 15 days of exposures the percent liver/body weight ratios were 1.67- and 1.12-fold of control for 5.0 and 0.5 g/L DCA and at 30 days were 1.99- and 1.16-fold, respectively. The difference in magnitude of dose and percent liver/body weight increase is difficult to determine given that the 5 g/L dose of DCA reduced body weight and significantly reduced water consumption by ~50%. The differences in DCA-induced percent liver/body weights were ~6-fold for the 15, 25, and 30-day data between the 0.5 and 5 g/L DCA exposures rather than the 10-fold difference in exposure concentration in the drinking water.

Parrish et al. (1996) reported that for male B6C3F1 mice exposed to TCA or DCA (0, 0.01, 0.5, and 2.0 g/L) for 3 or 10 weeks, the 4- to 5-fold magnitude of difference in doses resulted in increases in percent liver/body weight for the 21-day and 71-day exposures that were greater for DCA than TCA. The percent liver/body weight ratio were 0.98-, 1.13-, and 1.33-fold of control levels at 0.1, 0.5, and 2.0 g/L TCA and for DCA were 1.02-, 1.24-, and 1.46-fold of control levels, respectively, after 21 days of exposure. Both TCA and DCA exposures at 0.1 g/L resulted in difference in percent liver/body weight change of 2% or less. For TCA, although there was a 4-fold increase in magnitude between the 0.5 and 2.0 g/L TCA exposure concentrations, the magnitude of increase for percent liver/body weight increase was 2.5-fold between them at both 21 and 71 days of exposure. For DCA, the 4-fold difference in dose between the 0.5 and 2.0 g/L DCA exposure concentrations were reported to result in a ~2-fold increase in percent liver/body weight increase at 21 days and ~4.5-fold increase at 71 days.

DeAngelo et al. (2008) studied 3 exposure concentrations of TCA in male B6C3F1 mice, which were an order of magnitude apart, for 4 weeks of exposure. The percent liver/body weight ratios were 1.09-, 1.16-, and 1.35-fold of control levels, for 0.05, 0.5, and 5.0 g/L TCA exposures, respectively. The 10-fold differences in exposure concentration of TCA resulted in ~2-fold differences in percent liver/body weight increases. No dose-response inferences can be drawn from the 4-week study of DCA and TCA in B6C3F1 male mice by Kato-Weinstein et al. (2001) but 2 g/L DCA and 3 g/L TCA in drinking water were reported to induce percent liver/body weights of 1.42- and 1.33-fold of control, respectively ($n = 5$).

The majority of short-term studies of DCA and TCA in mice have been conducted in the B6C3F1 strain and in males. Studies conducted from 14 to 30 days show a consistent increase in percent liver/body weight induction by TCA or DCA. Accordingly an examination of all of the data from Parrish et al. (1996), Sanchez and Bull (1990), Carter et al. (1995), Kato-Weinstein et al. (2001), and DeAngelo et al. (1989, 2008) from 14 to 30 days of exposure in male B6C3F1 mice can give an approximation of the dose-response differences between DCA and TCA for liver weight induction as shown in Table E-14 and Figure E-1, below. Although the data for B6C3F1 mice from Sanchez and Bull (1990) is reported as the fold of liver weight rather that percent liver/body weight increase, it is included in the comparison as both reflect increase in liver weight. Similar data can be assessed for TCE for comparative purposes. Short duration studies (10−42 days) were selected because (1) in chronic studies, liver weight increases are confounded by tumor burden, (2) multiple studies are available, and (3) in this duration range, Kjellstrand et al. (1981) reported that TCE-induced increases in liver weight plateau, and (4) TCA studies do not show significant duration-dependent differences in this duration range. These comparisons are presented in Table E-14.

DeAngelo et al. (1989) and Carter et al. (1995) used up to 5 g/L DCA and TCA in their experiments with Carter et al. (1995) noting a dramatic decrease in water consumption in the 5 g/L DCA treatment groups (46−64% reduction) which can affect body weight as well as dose received. DeAngelo et al. (1989) did not report drinking water consumption. The drinking water consumption was reported by DeAngelo et al. (2008) to be reduced by 11, 17, and 30% in the 0.05, 0.5, and 5 g/L TCA treated groups compared to 2 g/L NaCl control animals over 60 weeks. DeAngelo et al. (1999) reported mean drinking water consumption to be reduced by 26% in mice exposed to 3.5 g/L DCA over 100 weeks. Carter et al. (1995) reported that DCA at 5 g/L to decrease drinking water consumption by 64 and 46% but 0.5 g/L DCA to not affect drinking water consumption. Thus, it appears that the 5 g/L concentrations of either DCA or TCA can significantly affect drinking water consumption as well as inducing reductions in body weight. Accordingly, an estimation of the shape of the dose-response curve for comparative purposes between DCA or TCA drinking water studies is best examined at concentrations at 2 g/L or less, especially for DCA.

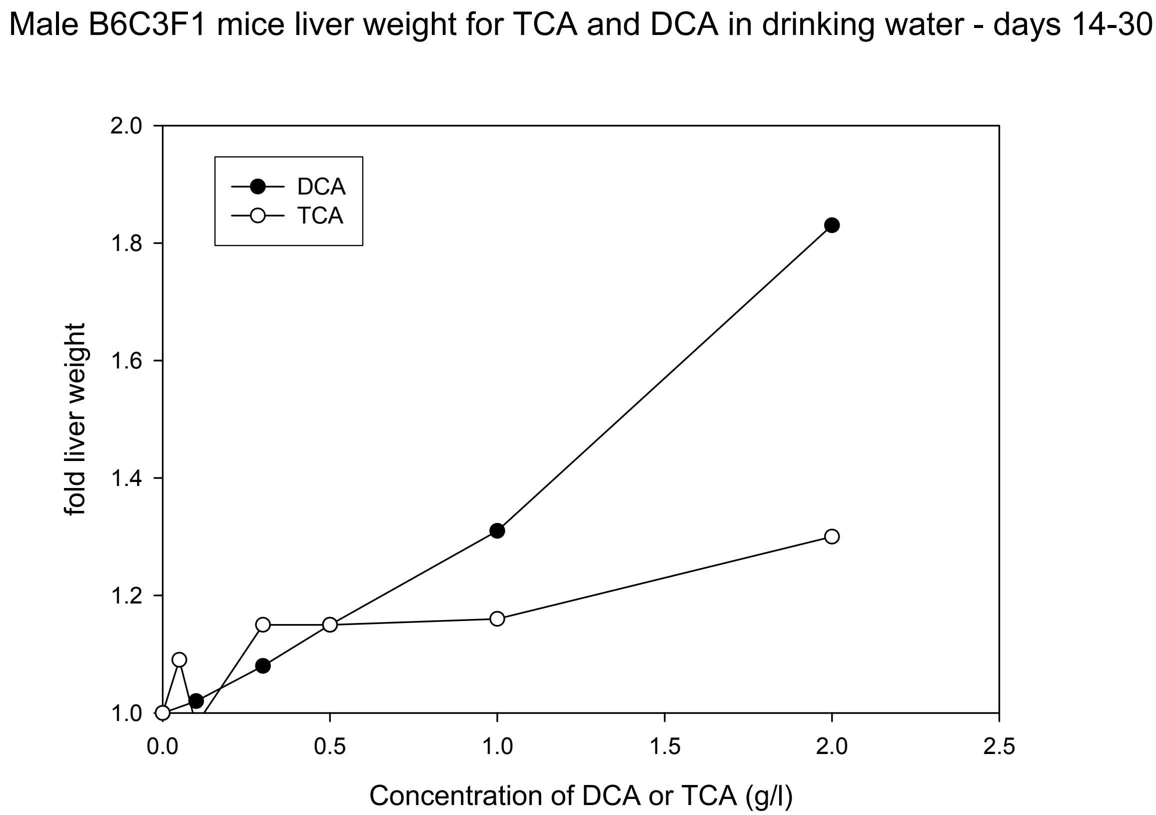
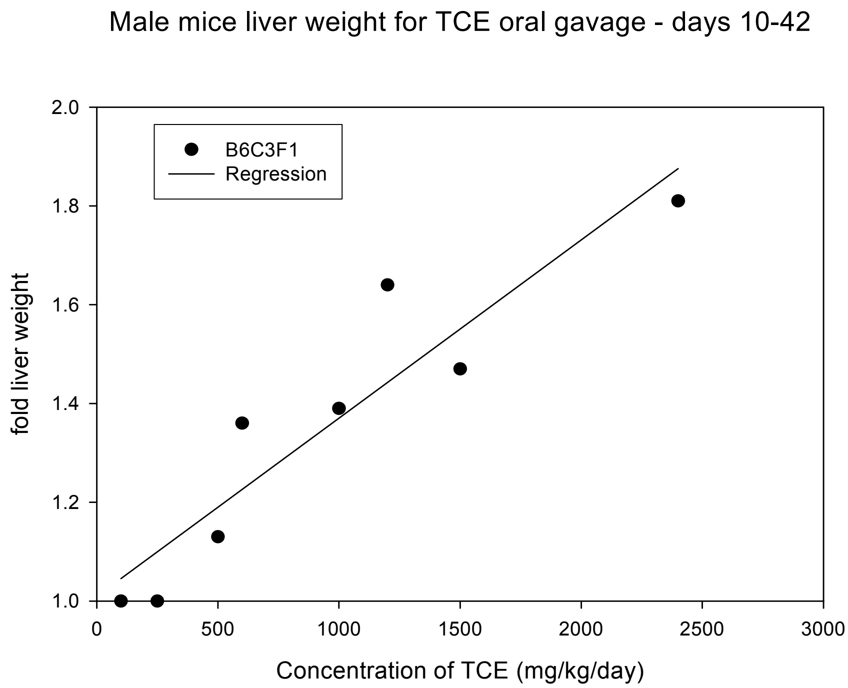


Figure E-1. Comparison of average fold-changes in relative liver weight to control and exposure concentrations of 2 g/L or less in drinking water for TCA and DCA in male B6C3F1 mice for 14−30 days (Parrish et al.,1996; Sanchez and Bull, 1990; Carter et al., 1995; Kato-Weinstein et al., 2001; DeAngelo et al., 1989, 2008). (Reproduced from Section 4.5.)

The dose-response curves for similar concentrations of DCA and TCA are presented in Figure E-1 for durations of exposure from 14−28 days in the male B6C3F1 mouse, which was the most common sex and strain used. For this comparative analysis an average is provided between two values for a given concentration and duration of exposure for comparison with other doses and time points. As noted in the discussion of individual experiments, there appears to be a linear correlation between dose in drinking water and liver weight induction up to 2 g/L of DCA. However, the shape of the dose-response curve for TCA appears to be quite different (i.e., lower concentrations of TCA inducing larger increase that does DCA but then the response reaching an apparent plateau for TCA at higher doses while that of DCA continues to increase). As shown by DeAngelo et al. (2008), 10-fold differences in the magnitude of exposure concentration to TCA corresponded to ~2-fold differences in liver weight induction increases. In addition, TCA studies did not show significant duration-dependent difference in liver weight induction in this duration range as shown in Table E-14.

Of interest is the issue of how the dose-response curves for TCA and DCA compare to that of TCE in a similar model and dose range. Since TCA and DCA have strikingly different dose-response curves, which one if either best fits that of TCE and thus, can give insight as to which is causative agent for TCE's effects in the liver? In the case of the TCE database in the mouse two strains have been predominantly studied, Swiss and B6C3F1, and both have been reported to get liver tumors in response to chronic TCE exposure. Rather than administered in drinking water, oral TCE studies have been conducted via oral gavage and generally in corn oil for 5 days of exposure per week. The study by Goel et al. (1992) was conducted in ground-nut oil. Vehicle effects, the difference between daily and weekly exposures, the dependence of TCE effects in the liver on its metabolism to a variety of agents capable inducing effects in the liver, differences in response between strains, and the inherent increased variability in use of the male mouse model all add to increased difficulty in establishing the dose-response relationship for TCE across studies and for comparisons to the DCA and TCA database. Despite difference in exposure route, etc., a consistent pattern of dose-response emerges from combining the available TCE data. The effects of oral exposure to TCE from 10−42 days on liver weight induction is shown in Figure E-2 using the data of Elcombe et al. (1985), Dees and Travis (1993), Goel et al. (1992), Merrick et al. (1989), Goldsworthy and Popp (1987), and Buben and O'Flaherty (1985). More detailed discussion of the 4- to 6-week studies is presented in Section E.2.4.3, below (e.g., for Merrick et al., 1989; Goel et al., 1992; Buben and O'Flaherty, 1985). For this comparative analysis an average is provided between two values per concentration and duration of exposure for comparison with other doses and time points. As shown by the 10-day data in B6C3 F1 mice, there are significant differences in response between studies of male B6C3F1 mice at the same dose of TCE. This variability is similar to findings from inhalation studies of TCE in male mice (Kjellstrand et al., 1983a).



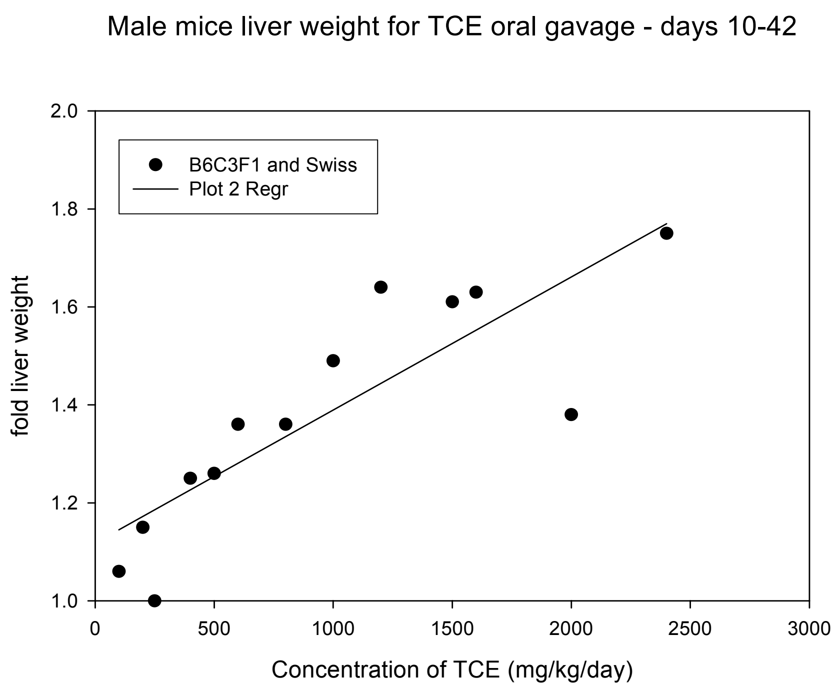


Figure E-2. Comparisons of fold-changes in average relative liver weight and gavage dose of (top panel) male B6C3F1 mice for 10−28 days of exposure (Merrick et al., 1989; Elcombe et al., 1985; Goldsworthy and Popp, 1987, Dees and Travis, 1993) and (bottom panel) in male B6C3F1 and Swiss mice. (Reproduced from Section 4.5.)

As shown in Figure E-2, oral TCE administration in male B6C3F1 and Swiss mice appeared to induce a dose-related increase in percent liver/body weight that was generally proportional to the increase in magnitude of dose, though as expected, with more variability than observed for a similar exercise for DCA or TCA in drinking water. Common exposure concentrations between B6C3F1 and Swiss mice were 100, 500, 1,000, 1,500 and 2,400 mg/kg/d TCE which corresponded to a 5-, 2-, 1.5-, and 1.6-fold difference in the magnitude of dose. For the data from studies in B6C3 F1 mice, there was no increase reported at 100 mg/kg/d TCE but between 500 and 1,000, 1,000 and 1,500, and 1,500 and 2,400 mg/kg/d TCE the magnitude of difference in doses matched that of the magnitude of increase in percent liver/body weight (i.e., a 2.6-, 1.4-, and 1.7-fold increase in liver weight was matched by a 2-, 1.5-, and 1.6-fold increase in TCE exposure concentration at these exposure intervals). However, only 10-day was available for doses between 100 and 500 mg/kg in B6C3F1 mice and at the lower doses, a 10-day interval may have been too short for the increase in liver weight to have been fully expressed. The database for the Swiss mice, which has more data from 28 and 42 days of exposure, support this conclusion. At 28−42 days of exposure there was a much greater increase in liver weight from TCE exposure in Swiss mice than the 10-day data in B6C3F1 mice. In Figure E-2, the 10-day data are included for comparative purpose for the B6C3F1 data set and the Swiss and B6C3F1 data sets combined. Both the combined TCE data and that for only B6C3F1 mice shows a correlation with the magnitude of dose and magnitude of percent liver/body weight increase. The slope of the dose-response curves are both closer to that of DCA than TCA. The correlation coefficients for the linear regressions presented for the B6C3F1 data are $R^2 = 0.861$ and for the combined data sets is $R^2 = 0.712$. Comparisons of the slopes of the dose-response curves indicate that TCA is not responsible for TCE-induced liver effects. In this regression all data points were treated equally although some came from several sets of data and others did not. Of note is that the 2,000 mg/kg TCE data point in the combined data set, which is much lower in liver weight response than the other data, is from one experiment (Goel et al., 1992), from 6 mice, at one time point (28 days), and one strain (Swiss). Deletion of these data point from the rest of the 23 used in the study results in a better fit to the data of the regression analysis.

A more direct comparison would be on the basis of dose rather than drinking water concentration. The estimations of internal dose of DCA or TCA from drinking water studies have been reported to vary with DeAngelo et al. reporting DCA drinking water concentrations of 1.0, 2.0, and 5.0 g/L to result in 90, 166, and 346 mg/kg/d, respectively. For TCA, 0.05, 0.5, 1.0, 2.0, and 5 g/L drinking water exposures were reported to result in 5.8 (range 3.6−8.0), 50 (range of 32.5 to 68), 131, 261, and 469 (range 364 to 602) mg/kg/d doses. The estimations of internal dose of DCA or TCA from drinking water studies, while varying considerably (DeAngelo et al., 1989, 2008), nonetheless suggest that the doses of TCE used in the gavage experiments were much higher than those of DCA or TCA. However, only a fraction of ingested TCE is metabolized to DCA or TCA, as, in addition to oxidative metabolism, TCE is also cleared by glutathione (GSH) conjugation and by exhalation.

While DCA dosimetry is highly uncertain (see Sections E.3.3 and E.3.5), the mouse physiologically based pharmacokinetic (PBPK) model, described in Section E.3.5 was calibrated using extensive *in vivo* data on TCA blood, plasma, liver, and urinary excretion data from inhalation and gavage TCE exposures, and makes robust predictions of the rate of TCA production. If TCA were predominantly responsible for TCE-induced liver weight increases, then replacing administered TCE dose (e.g., mg TCE/kg/day) by the rate of TCA produced from TCE (mg TCA/kg/day) should lead to dose-response curves for increased liver weight consistent with those from directly administered TCA. Figure E-3 shows this comparison using the PBPK model-based estimates of TCA production for 4 TCE studies from 28−42 days in the male NMRI, Swiss, and B6C3F1 mice (Kjellstrand et al., 1983b; Buben and O'Flaherty, 1985; Merrick et al., 1989; Goel et al., 1992) and 4 oral TCA studies in B6C3F1 male mice at 2 g/L or lower drinking water exposure (DeAngelo et al., 1989, 2008; Parrish et al., 1996; Kato-Weinstein et al., 2001) from 14−28 days of exposure. The selection of the 28−42 day data for TCE was intended to address the decreased opportunity for full expression of response at 10 days. PBPK modeling predictions of daily internal doses of TCA in terms of mg/kg/d via produced via TCE metabolism would be are indeed lower than the TCE concentrations in terms of mg/kg/d given orally by gavage. The predicted internal dose of TCA from TCE exposure studies are of a comparable range to those predicted from TCA drinking water studies at exposure concentrations in which palability has not been an issue for estimation of internal dose. Thus, although the TCE data are for higher exposure concentrations, they are predicted to produce comparable levels of TCA internal dose estimated from direct TCA administration in drinking water.

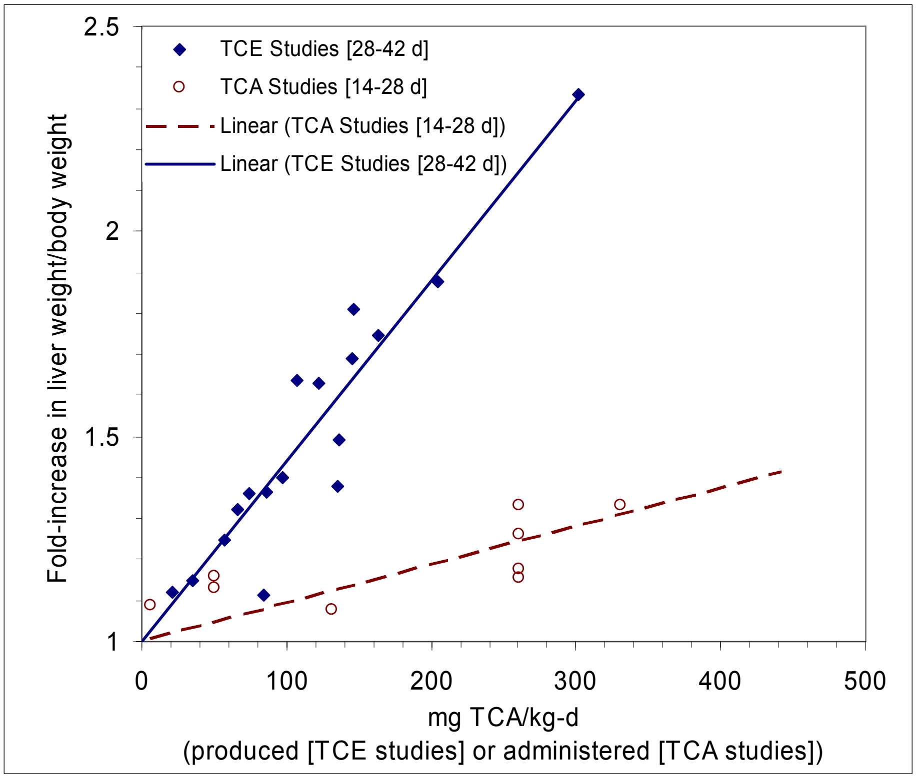


Figure E-3. Comparison of fold-changes in relative liver weight for data sets in male B6C3F1, Swiss, and NRMI mice between TCE studies (Kjellstrand et al., 1983b; Buben and O'Flaherty, 1985; Merrick et al., 1989; Goel et al., 1992) [duration 28−42 days] and studies of direct oral TCA administration to B6C3 F1 mice (DeAngelo et al., 1989; Parrish et al., 1996; Kato-Weinstein et al., 2001; DeAngelo et al., 2008) [duration 14−28 days]. Abscissa for TCE studies consists of the median estimates of the internal dose of TCA predicted from metabolism of TCE using the PBPK model described in Section 3.5 of the TCE risk assessment. Lines show linear regression with intercept fixed at 1. All data were reported fold-change in mean liver weight/body weight ratios, except for Kjellstrand et al. (1983b), with were the fold-change in the ratio of mean liver weight to mean body weight. In addition, in Kjellstrand et al. (1983b), some systemic toxicity as evidence by decreased total body weight was reported in the highest dose group. (Reproduced from Section 4.5.)

Figure E-3 clearly shows that for a given amount of TCA produced from TCE, but going through intermediate metabolic pathways, the liver weight increases are substantially greater than, and highly inconsistent with, that expected based on direct TCA administration. In particular, the response from direct TCA administration appears to "saturate" with increasing TCA dose at a level of about 1.4-fold, while the response from TCE administration continues to increase with dose to 1.75-fold at the highest dose administered orally in Buben and O'Flaherty (1985) and over 2-fold in the inhalation study of Kjellstrand et al. (1983b). For this analysis is unlikely that strain differences can account for this inconsistency in the dose-response curves. TCE-induced increases in liver weight appear to be generally similar between B6C3F1 and Swiss male mice (see Table E-14) via oral exposure and between NMRI male and female mice after inhalation, although the NMRI strain appeared to be more prone to TCE-induced toxicity in male mice and for females to have a smaller TCE-induced liver weight increase than other strains (Kjellstrand et al., 1983b). As noted previously, the difference in response between strains and between studies in the same strain for TCE liver weight increases can be highly variable. Little data exist to examine this issue for TCA studies although DeAngelo et al. (1989) report a range of 1.16- to 1.63-fold of control percent liver/body weight increase after 14 days exposure at 2 g/L TCA in the Swiss-Webster, C3H, C57BL/6, and B6C3F1 strains, with differences also noted between 2 studies of the B6C3F1 mouse.

Furthermore, while as noted previously, oral studies appear to report a linear relationship between TCE exposure concentration and liver weight induction, the inclusion of inhalation studies on the basis of internal dose led to a highly consistent dose-response curve for among TCE study. Therefore, it is unlikely that differing routes of exposure can explain the inconsistencies in dose-response. The PBPK model predicted that matching average TCA production by TCE with the equivalent average dose from drinking water-administered TCA also led to an equivalent area-under-the-curve (AUC) of TCA in the liver. Moreover, Dees and Travis (1993) administered 100 to 1,000 mg/kg/d TCA by gavage to male and female B6C3F1 mice for 11 days, and did not observe increases in liver/body weight ratios more than 1.28-fold, no higher than those observed with drinking water exposures. Finally, the dose-response consistency between TCE inhalation and gavage studies argues against route of exposure significantly impacting liver weight increases. Thus, no level of TCA administration appears able account for the continuing increase in liver weights observed with TCE, quantitatively inconsistent with TCA being the predominant metabolite responsible for TCE-induced liver weight changes. Thus, involvement of other metabolites, besides TCA, is implicated as the causes of TCE-induced liver effects.

Additional analyses do, however, support a role for oxidative metabolism in TCE-induced liver weight increases, and that the parent compound TCE is not the likely active moeity (suggested previously by Buben and O’Flaherty [1985]). In particular, the same studies are shown in Figure E-4 using PBPK-model based predictions of the AUC of TCE in blood and total oxidative metabolism, which produces chloral, trichloroethanol, DCA, and other metabolites in addition to TCA. The dose-response relationship between TCE blood levels and liver weight increase, while still having a significant trend, shows substantial scatter and a low R^2 of 0.43. On the other hand, using total oxidative metabolism as the dose metric leads to substantially more consistency dose-response across studies, and a much tighter linear trend with an R^2 of 0.90 (see Figure E-4). A similar consistency is observed using liver-only oxidative metabolism as the dose metric, with R^2 of 0.86 (not shown). Thus, while the slope is similar between liver weight increase and TCE concentration in the blood and liver weight increase and rate of total oxidative metabolism, the data are a much better fit for total oxidative metabolism.

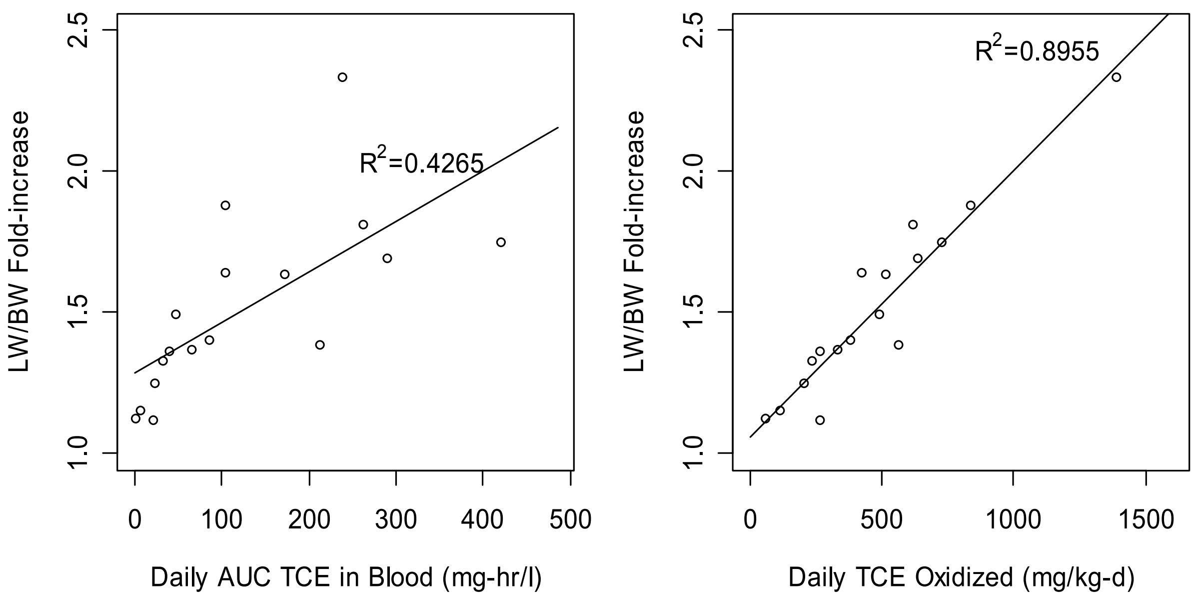


Figure E-4. Fold-changes in relative liver weight for data sets in male B6C3F1, Swiss, and NRMI mice reported by TCE studies of duration 28−42 days (Kjellstrand et al., 1983b; Buben and O'Flaherty, 1985; Merrick et al., 1989; Goel et al., 1992) using internal dose metrics predicted by the PBPK model described in Section E.3.5: (A) dose metric is the median estimate of the daily AUC of TCE in blood, (B) dose metric is the median estimate of the total daily rate of TCE oxidation. Lines show linear regression. Use of liver oxidative metabolism as a dose metric gives results qualitatively similar to (B), with R^2 = 0.86. (Reproduced from Section 4.5.)

As stated in many of the discussions of individual studies, there is a limited ability to detect a statistically significant change in liver weight change in experiments that use a relatively small number of animals. Many experiments have been conducted with 4−6 mice per dose group. The experiments of Buben and O'Flaherty used 12−14 mice per group giving it a greater ability to detect a TCE-induced dose response. In some experiments greater care was taken to document and age and weight match the control and treatment groups before the start of treatment. The approach taken above for the analyses of TCE, TCA and DCA uses data across several data sets and gives a more robust description of these dose-response curves, especially at lower exposure levels. For example, the data from DeAngelo et al. (2008) for TCA-induced percent liver/body weight ratio increases in male B6C3F1 mice were only derived from 5 animals per treatment group after 4 weeks of exposure. The 0.05 and 0.5 g/L exposure concentrations were reported to give a 1.09- and 1.16-fold of control percent liver/body weight ratios, which were consistent with the increases noted in the cross-study database above. However, a power calculation shows that the type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was only a 6 and 7% and therefore, the designed experiment could accept a false null hypothesis.

Although the qualitative similarity to the linear dose-response relationship between DCA and liver weight increases is suggestive of DCA being the predominant metabolite responsible for TCE liver weight increases, due to the highly uncertain dosimetry of DCA derived from TCE, this hypothesis cannot be tested on the basis of internal dose. Similarly, another TCE metabolite, CH, has also been reported to induce liver tumors in mice, however, there are no adequate comparative data to assess the nature of liver weight increases induced by this TCE metabolite (see Section E.2.5, below). Whether its formation in the liver after TCE exposure correlates with TCE-induced liver weight changes cannot be determined. Of note is the high variability in total oxidative metabolism reported in mice and humans of Section 3.3, which suggests that the correlation of total TCE oxidative metabolism with TCE-induced liver effects should lead not only to a high degree of variability in response in rodent bioassays which is the case (see Section E.2.4.4, below) but also make detection of liver effects more difficult in human epidemiological studies (see Section 4.3.2). What mechanisms or events are leading to liver weight increases for DCA, TCA and TCE can be examined by correlations between changes in glycogen content, hepatocyte volume, and evidence of polyploidization noted in short-term assays.

Data have been reported regarding the nature of changes the TCE and its metabolites induce in the liver and are responsible for the reported increases in liver weight. Increased liver weight may result from increased size or hypertrophy of hepatocytes through changes in glycogen deposition, but also through increased polyploidization. Increased cell number may also contribute to increased liver weight. As noted above in Section E.2.4.1, hepatocellular hypertrophy appeared to be related to TCE-induced liver weight changes after short-term exposures. However, neither glycogen deposition, DNA synthesis, or increases in mitosis appear to be correlated with liver weight increases. In particular DNA synthesis increases were similar from 250−1,000 mg/kg and peroxisomal volume was similar between 500 and 1,500 mg/kg TCE exposures after 10 days. Autoradiographs identified hepatocytes undergoing DNA synthesis in "mature" hepatocytes that were in areas where polyploidization typically takes place in the liver.

By 14 days of exposure, Sanchez and Bull (1990) reported that both dose-related TCA- and DCA-induced increases in liver weight were generally consistent with changing cell size increases, but were not correlated with patterns of change in hepatic DNA content, incorporation of tritiated thymidine in DNA extracts from whole liver, or incorporation of tritiated thymidine in hepatocytes. There are conflicting reports of DNA synthesis induction in individual hepatocytes for up to 14 days of DCA or TCA exposure and a lack of correlation with patterns observed for this endpoint and those of whole liver thymidine incorporation. The inconsistency of whole liver DNA tritiated thymidine incorporation with that reported for hepatocytes was noted by the Sanchez and Bull (1990) to be unexplained. Carter et al. (1995) also report a lack of correlation between hepatic DNA tritiated thymidine incorporation and labeling in individual hepatocytes in male mice. Carter et al. (1995) reported no increase in labeling of hepatocytes in comparison to controls for any DCA treatment group from 5 to 30 days of DCA exposure. Rather than increase hepatocyte labeling, DCA induced a decrease with no change reported from days 5 though 15 but significantly decreased levels between days 20 and 30 for 0.5 g/L that were similar to those observed for the 5 g/L exposures.

The most comparable time period between TCE, TCA and DCA results for whole liver thymidine incorporation is the 10- and 14-day durations of exposure when peak tritiated thymidine incorporation into individual hepatocytes and whole liver for TCA and DCA have been reported to have already passed (Styles et al., 1991; Sanchez and Bull, 1990; Pereira, 1996; Carter et al., 1995). Whole liver DNA synthesis was elevated over control levels by ~2-fold after from 250 to 1,000 mg/kg TCE exposure after 10 days of exposure but did not correlate with mitosis (Elcombe et al., 1985; Dees and Travis, 1993). After 3 weeks of exposure to TCE, Laughter et al. (2004) reported in individual hepatocytes that 1 and 4.5% of hepatocytes had undergone DNA synthesis in the last week of treatment for the 500 and 1,000 mg/kg TCE levels, respectively. More importantly, these data show that hepatocyte proliferation in TCE-exposed mice at 10 days of exposure or for DCA- or TCA-exposed mice for up to 14 days of exposure is confined to a very small population of cells in the liver.

In regard to cell size, although increased glycogen deposition with DCA exposure was noted by Sanchez and Bull (1990), lack of quantitative analyses of that accumulation in this study precludes comparison with DCA-induced liver weight gain. Although not presenting a quantitative analysis, Sanchez and Bull (1990) reported DCA-treated B6C3F1 mice to have large amounts of PAS staining material and Swiss-Webster mice to have similar increase despite reporting differences of DCA-induced liver weight gain between the two strains. The lack of concordance of the DCA-induced magnitude of increase in liver weight with that of glycogen deposition is consistent with the findings for longer-term exposures to DCA reported by Kato-Weinstein et al. (2001) and Pereira et al. (2004) in mice (see Section E.2.4.4, below). Carter et al. (1995) reported that in control mice there was a large variation in apparent glycogen content and also did not perform a quantitative analysis of glycogen deposition. The variability of this parameter in untreated animals and the extraction of glycogen during normal tissue processing for light microscopy makes quantitative analyses for dose-response difficult unless specific methodologies are employed to quantitatively assess liver glycogen levels as was done by Kato-Weinstein et al. (2001) and Pereira et al. (2004).

Although suggested by their data, polyploidization was not examined for DCA or TCA exposure in the study of Sanchez and Bull (1990). Carter et al. (1995) reported that hepatocytes from both 0.5 and 5 g/L DCA treatment groups were reported to have enlarged, presumably polyploidy nuclei with some hepatocyte nuclei labeled in the mid-zonal area. There were statistically significant changes in cellularity, nuclear size, and multinucleated cells during 30 days exposure to DCA. The percentage of mononucleated cells hepatocytes was reported to be similar between control and DCA treatment groups at 5- and 10-day exposure. However, at 15 days and beyond, DCA treatments were reported to induce increases in mononucleated hepatocytes. At later time periods there were also reports of DCA-induced increases nuclear area, consistent with increased polyploidization without mitosis. The consistent reporting of an increasing number of mononucleated cells between 15 and 30 days could be associated with clearance of mature hepatocytes as suggested by the report of DCA-induced loss of cell nuclei. The reported decrease in the numbers of binucleate cells in favor of mononucleate cells is not typical of any stage of normal liver growth (Brodsky and Uryvaeva, 1977). The linear dose-response in DCA-induced liver weight increase was not consistent with the increased numbers of mononucleate cells and increase nuclear area reported from Day 20 onward by Carter et al. (1995). Specifically, the large differences in liver weight induction between the 0.5 g/L treatment group and the 5 g/L treatment groups at all times studied also did not correlate with changes in nuclear size and percent of mononucleate cells. Thus, DCA-induced increases in liver weight were not a function of cellular proliferation, but probably included hypertrophy associated with polyploidization, increased glycogen deposition and other factors.

In regard to necrosis, Elcombe et al. (1985) reported only small incidence of focal necrosis in 1,500 mg/kg TCE-exposed mice and no necrosis at exposures up to 1,000 mg/kg for 10 days as did Dees and Travis (1993). Sanchez and Bull (1990) report DCA-induced localized areas of coagulative necrosis both for B6C3F1 and Swiss-Webster mice at higher exposure levels (1 or 2 g/L) by 14 days but not at the 0.3 g/L level or earlier time points. For TCA treatment, necrosis was reported to not be associated with TCA treatment for up to 2 g/L and up to 14 days of exposure. Carter et al. (1995) reported that mice given 0.5 g/L DCA for 15, 20, and 25 days had midzonal focal cells with less detectable or no cell membranes, loss of the coarse granularity of the cytoplasm, with some cells having apparent karyolysis, but for liver architecture to be normal.

As for apoptosis, Both Elcombe et al. (1985) and Dees and Travis (1993) reported no changes in apoptosis other than increased apoptosis only at a treatment level of 1,000 mg/kg TCE. Rather than increases in apoptosis, peroxisome proliferators have been suggested to inhibit apoptosis as part of their carcinogenic MOA (see Section E.3.4.1). However, the age and species studied appear to greatly affect background rates of apoptosis. Snyder et al. (1995) report that control mice were reported to exhibit apoptotic frequencies ranging from ~0.04 to 0.085%, that over the 30-day period of their study the frequency rate of apoptosis declined, and suggest that this pattern is consistent with reports of the livers of young animals undergoing rapid changes in cell death and proliferation. They reported rat liver to have a greater the estimated frequency of spontaneous apoptosis (~0.1%) and therefore, greater than that of the mouse. Carter et al. (1995) reported that after 25 days of 0.5 g/L DCA treatment apoptotic bodies were reported as well as fewer nuclei in the pericentral zone and larger nuclei in central and midzonal areas. This would indicate an increase in the apoptosis associated potential increases in polyploidization and cell maturation. However, Snyder et al. (1995) report that mice treated with 0.5 g/L DCA over a 30-day period had a similar trend as control mice of decreasing apoptosis with age. The percentage of apoptotic hepatocytes decreased in DCA-treated mice at the earliest time point studied and remained statistically significantly decreased from controls from 5 to 30 days of exposure. Although the rate of apoptosis was very low in controls, treatment with 0.5 g/L DCA reduced it further (~30−40% reduction) during the 30-day study period. The results of this study not only provide a baseline of apoptosis in the mouse liver, which is very low, but also to show the importance of taking into account the effects of age on such determinations. The significance of the DCA-induced reduction in apoptosis reported in this study, from a level that is already inherently low in the mouse, to account for the MOA for induction of DCA-induced liver cancer is difficult to discern.

E.2.4.3. *Summary Trichloroethylene (TCE) Subchronic and Chronic Studies*

The results of longer-term (Channel et al., 1998; Toraason et al., 1999; Parrish et al., 1996) studies of "oxidative stress" for TCE and its metabolites are discussed in Section E.3.4.2.3. Of note are the findings that the extent of increased enzyme activities associated with peroxisome proliferation do not appear to correlate with measures of oxidative stress after longer term exposures (Parrish et al., 1996) and single strand breaks (Chang et al., 1992).

Similar to the reports of Melnick et al. (1987) in rats, Merrick et al. (1989) report that vehicle (aqueous or gavage) affects TCE-induced toxicity in mice. Vehicle type made a large difference in mortality, extent of liver necrosis, and liver weight gain in male and female B6C3F1 mice after 4 weeks of exposure. The lowest dose used in this experiment was 600 mg/kg/d in males and 450 mg/kg/d in females. Administration of TCE via gavage using Emulphor resulted in mortality of all of the male mice and most of the female mice at a dose in corn oil that resulted in few deaths. However, use of Emulphor vehicle induced little if any focal necrosis in males at concentrations of TCE in corn oil gavage that caused significant focal necrosis, indicating vehicle effects.

As discussed above in Section E.2.4.2, the extent of TCE-induced liver weight increases was consistent between 4 and 6 weeks of exposure and between 10-day and 4 week exposure at higher dose levels. In general, the reported elevations of enzymatic markers of liver toxicity and results for focal hepatocellular necrosis were not consistent and did not reflect TCE dose-responses observed for induction of liver weight increases (Merrick et al., 1989). Female mice given corn oil and male and female mice given TCE in Emulphor were reported to have "no to negligible necrosis" although they had increased liver weight from TCE exposure. Using a different type of oil vehicle, Goel et al. (1992) exposed male Swiss mice to TCE in groundnut oil at concentrations ranging from 500 to 2,000 mg/kg for 4 weeks and reported no changes in body weight up to 2,000 mg/kg, although there was a 15% decrease at the highest dose, but increases TCE-induced increase in percent liver/body weight ratio. At a dose of 1,000 and 2,000 mg/kg, liver swelling, vacuolization, and widespread degenerative necrosis of hepatocytes was reported along with marked proliferation of "endothelial cells" but no quantitation regarding the extent or location of hepatocellular necrosis was reported, nor whether there was a dose-response relationship in these events. They reported a TCE-related dose-response in catalase, liver protein but decreased induction at the 2,000 mg/kg level where body weight had decreased.

Three studies were published by Kjellstrand et al. that examined effects of TCE inhalation primarily in mice using whole body inhalation chambers (Kjellstrand et al., 1981, 1983a, b). Liver weight changes were used as the indication of TCE-induced effects. The quantitative results from these experiments had many limitations due to their experimental design including failure to determine body weight changes for individual animals and inability to determine the exact magnitude of TCE due to concurrent oral TCE ingestion from food and grooming behavior. An advantage of this route of exposure is that there were not confounding vehicle effects. The results from Kjellstrand et al. (1981) are particularly limited by experimental design errors but reported similar increases in liver weight gain in gerbils and rats exposed at 150 ppm TCE. For rats, Kjellstrand et al. (1981) do report increases in liver/body weight ratios of 1.26- and 1.21-fold of control in male and female rat 30 days of continuous TCE inhalation exposure. The unpublished report of Woolhiser et al. (2006) reports 1.05-, 1.07-, and 1.13-fold of control percent liver/body weight changes in 100-, 300- and 1,000-ppm-exposure groups that are exposed for 6 hours/day, 5 days/week for 4 weeks in groups of 8 female S-D rats. At the two highest exposure levels, body weight was reduced by TCE exposure. If the 150 ppm continuous exposure concentrations of Kjellstrand are analogous to 750–ppm-exposures using the paradigm of Woolhiser et al. (2006). Therefore, the very limited inhalation database for rats does indicate TCE-related increases in liver weight.

The study of Kjellstrand et al. (1983a) employed a more successful experimental design that recorded liver weight changes in carefully matched control and treatment groups to determine TCE-treatment related effects on liver weight in 7 strains of mice after 30 days of continuous inhalation exposure at 150 ppm TCE. Individual animal body weight changes were not recorded so that such an approach cannot take into account the effects of body weight changes and determine a relative percent liver/body weight ratio. The data presented in this report was for absolute liver weight changes between treated and nontreated groups with carefully matched average body weights at the initiation of exposure. A strength of the experimental design is its presentation of results between duplicate experiments and thus, to show the differences in results between similar exposed groups that were conducted at different times. This information gives a measure of variability in response with time. Mouse strain groups, that did not experience TCE-induced decreased body weight gain in comparison to untreated groups (i.e., DBA and wild-type mice), represented the most accurate determination of TCE-induced liver weight changes given that systemic toxicity that affects body weight can also affect liver weight. The C57BL, B6CBA, and NZB groups all had at least one group out of two of male mice with changes in final body weight due to TCE exposure. Only one group of NMRI mice were reported in this study and that group had TCE-induced decreases in final body weight. The A/sn group not only had both male groups with decreased final body weight after TCE exposure (along with differences between exposed and control groups at the initiation of exposure) but also a decrease in body weight in one of the female groups and thus, appears to be the strain with the greatest susceptibility to TCE-induced systemic toxicity. In strains of male mice in which there was no TCE-induced affects on final body weight (wild-type and DBA), the influence of gender on liver weight induction and variability of the response could be more readily assessed. In wild-type mice there was a 1.76- and 1.80-fold of control liver weight in groups 1 and 2 for female mice, and for males a 1.84- and 1.62-fold of control liver weight for groups 1 and 2, respectively. For DBA mice there was a 1.87- and 1.88-fold of control liver weight in groups 1 and 2 for female mice, and for males a 1.45- and 2.00-fold of control liver weight for groups 1 and 2, respectively. Of note, as described previously, the size of the liver is under strict control in relation to body size. An essential doubling of the size of the liver is a profound effect with the magnitude of liver weight size increase physiologically limited.

Overall, the consistency between groups of female mice of the same strain for TCE-induced liver weight gain, regardless of strain examined, was striking as was the lack of body weight changes at TCE exposure levels that induced body weight changes in male mice. In the absence of body weight changes, the difference in TCE-response in female mice appeared to be reflective of strain and initial weight differences. Groups of female mice with higher body weights, regardless of strain, generally had higher increases in TCE-induced liver weight increases. For the C57BL and As/n strains, female mice starting weights were averaged 17.5 and 15.5 g, while the average liver weights were 1.63- and 1.64-fold of control after TCE exposure, respectively. For the B6CBA, wild-type, DBA, and NZB female groups the starting body weights averaged 22.5, 21.0, 23.0, and 21.0 g, while the average liver weights were 1.70-, 1.78-, 1.88-, and 2.09-fold of control after TCE exposure, respectively. The NMRI group of female mice, did not follow this general pattern and had the highest initial body weight for the single group of 10 mice reported (i.e., 27 g) associated with 1.66-fold of control liver weight.

The results of Kjellstrand et al. (1983a) suggested that there was more variability between male mice than female mice in relation to TCE-induced liver weight gain. More strains exhibited TCE-induced body weight changes in male mice than female mice suggesting increased susceptibility of male mice to TCE toxicity as well as more variability in response. Initial body weight also appeared to be a factor in the magnitude of TCE-induced liver weight induction rather than just strain. In general, the strains and groups within strain that had TCE-induced body weight decreases had smaller TCE-induced increase in liver weight. Therefore, only examining liver weight in males as an indication of TCE treatment effects would not be an accurate predictor of strain sensitivity nor the magnitude or response at doses that also affect body weight. The results from this study show that comparison of the magnitude of TCE response, as measured by liver weight increases, should take into account, strain, gender, initial body weight and systemic toxicity. It shows a consistent pattern of increased liver weight in both male and female mice after TCE exposure of 150 ppm for 30 days.

Kjellstrand et al. (1983b) presented data in the NMRI strain of mice (a strain that appeared to be more prone to TCE-induced toxicity in male mice and a smaller TCE-induced increase in liver weight in female mice) after inhalation exposure of 37 to 300 ppm TCE. They used the same experimental paradigm as that reported in Kjellstrand et al. (1983a) except for exposure concentration. For female mice exposed to concentrations of TCE ranging from 37 to 300 ppm TCE continuously for 30 days, only the 300 pm group experienced a 16% decrease in body weight between control and exposed animals and therefore, changes in TCE-induced liver weight increases were affected by changes in body weight only for that group. Initial body weights in the TCE-exposed female mice were similar in each of these groups (i.e., range of 29.2−31.6 g, or 8%), with the exception of the females exposed to 150 ppm TCE for 30 days (i.e., initial body weight of 27.3 g), reducing the effects of differences in initial body weight on TCE-induced liver weight induction. Exposure to TCE continuously for 30 days was reported to result in a linear dose-dependent increase in liver weight in female mice with 1.06-, 1.27-, 1.66-, and 2.14-fold of control liver weights reported at 37 ppm, 75 ppm, 150 ppm, and 300 ppm TCE, respectively. In male mice there were more factors affecting reported liver weight increases from TCE exposure. For male mice both the 150- and 300-ppm-exposed groups experienced a 10 and 18% decrease in final body weight after TCE exposure, respectively. The 37- and 75-ppm groups did not have decreased final body weight due to TCE exposure but varied by 12% in initial body weight. TCE-induced increases in liver weight were reported to be 1.15-, 1.50-, 1.69-, and 1.90-fold of control for 37, 75, 150, and 300 ppm TCE exposure in male mice, respectively. The flattening of the dose-response curve at the two highest doses is consistent with the effects of toxicity on final body weight.

Kjellstrand et al. (1983b) noted that liver mass increase and the changes in liver cell morphology were similar in TCE-exposed male and female mice and report that after 150 ppm exposure for 30 days, liver cells were generally larger and often displayed a fine vacuolization of the cytoplasm, changes in nucleoli appearance, Kupffer cells of the sinusoid to be increased in cellular and nuclear size, the intralobular connective tissue was infiltrated by inflammatory cells and for exposure to TCE in higher or lower concentrations during the 30 days to produce a similar morphologic picture. For mice that were exposed to 150 ppm TCE for 30 days and then examined 120 days after the cessation of exposure, liver weights were 1.09-fold of control for TCE-exposed female mice and the same as controls for TCE-exposed male mice. However, the livers were not the same as untreated liver in terms of histopathology. The authors reported that "after exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes." The authors did not present any quantitative data on the lesions they describe, especially in terms of dose-response, and most of the qualitative description is for the 150–ppm-exposure level in which there are consistent reports of TCE induced body weight decreases in male mice. Although stating that Kupffer cells were increased in cellular and nuclear size, no differential staining was applied to light microscopy sections and used to distinguish Kupffer from endothelial cells lining the hepatic sinusoid in this study. Without differential staining such a determination is difficult at the light microscopic level and a question remains as to whether theses are the same cells as described by Goel et al. (1992) as a proliferation of sinusoidal endothelial cells after exposures of 1,000 and 2,000 mg/kg/d TCE exposure for 28 days in male Swiss mice. As noted in Section E.2.4.2, the discrepancy in DNA synthesis measures between hepatocyte examinations of individual hepatocytes and whole liver measures in several reports of TCE metabolite exposure, is suggestive of increased DNA synthesis in the nonparenchymal cell compartment of the liver. Thus, nonparenchymal cell proliferation is suggested as an effect of subchronic TCE exposures in mice without concurrent focal necrosis via inhalation studies (Kjellstrand et al., 1983b) and with focal necrosis in the presence of TCE in a groundnut oil vehicle (Goel et al., 1992).

Although Kjellstrand et al. (1983b) did not discuss polyploidization, the changes in cell size and especially the continued change in cell size and nuclear staining characteristics after 120 days of cessation of exposure are consistent with changes in polyploidization induced by TCE that were suggested in studies from shorter durations of exposure (Elcombe et al., 1985; Dees and Travis, 1993) and of longer durations (e.g., Buben and O'Flaherty, 1985). Of note is that in the histological description provided by Kjellstrand et al. (1983b), there is no mention of focal necrosis or apoptosis resulting from these exposures to TCE to mice. Vacuolization is reported and consistent with hepatotoxicity or lipid accumulation, which is lost during routine histological slide preparation. The lack of reported focal necrosis in mice exposed through inhalation is consistent with reports of gavage experiments of TCE in mice that do not use corn oil as the vehicle (Merrick et al., 1989).

Buben and O'Flaherty (1985) reported the effects of TCE via corn oil gavage after six weeks of exposure at concentrations ranging from 100 to 3,200 mg/kg d. This study was conducted with older mice than those generally used in chronic exposure assays (Male Swiss-Cox outbred mice between 3 and 5 months of age). Liver weight increases, decreases in liver G6P activity, increases in liver triglycerides, and increases in SGPT activity were examined as parameters of liver toxicity. Few deaths were reported during the 6-week exposure period except at the highest dose and related to central nervous system depression. TCE exposure caused dose-related increases in percent liver/body weight with a dose as low as 100 mg/kg/d were reported to cause a statistically significant increase (i.e., 112% of control). The increases in liver size were attributed to hepatocyte hypertrophy, as revealed by histological examination and by a decrease in the liver DNA concentration, and although enlarged, were reported to appear normal. A dose-related trend toward triglyceride concentration was also noted. A dose-related decrease in glucose-6-phophatase activity was reported with similar small decreases (~10%) observed in the TCE exposed groups that did not reach statistical significance until the dose reached 800 mg/kg TCE exposure. SGPT activity was not observed to be increased in TCE-treated mice except at the two highest doses and even at the 2,400 mg/kg dose half of the mice had normal values. The large variability in SGPT activity was indicative of heterogeneity of this response between mice at the higher exposure levels for this indicator of liver toxicity. Such variability of response in male mice is consistent with the work of Kjellstrand et al. Thus, the results from Buben and O'Flaherty (1985) suggest that hepatomegaly is a robust response that was reported to be observed at the lowest dose tested, dose-related, and not accompanied by overt toxicity.

In terms of histopathology, Buben and O'Flaherty (1985) reported swollen hepatocytes with indistinct borders; their cytoplasm was clumped and a vesicular pattern was apparent and not simply due to edema in TCE-treated male mice. Karyorhexis (the disintegration of the nucleus) was reported to be present in nearly all specimens from TCE-treated animals and suggestive of impending cell death, not present in controls, and to appear at a low level at 400 mg/kg TCE exposure level and slightly higher at 1,600 mg/kg TCE exposure level. Central lobular necrosis was present only at the 1,600 mg/kg TCE exposure level and at a very low level. Buben and O'Flaherty report increased polyploidy in the central lobular region for both 400 mg/kg and 1,600 mg/kg TCE and described as hepatic cells having two or more nuclei or enlarged nuclei containing increased amounts of chromatin, but at the lowest level of severity or occurrence. Thus, the results of this study are consistent with those of shorter-term studies via gavage, which report hepatocellular hypertrophy in the centralobular region, increased liver weight induced at the lowest exposure level tested and at a level much lower than those inducing overt toxicity, and that TCE exposure is associated with changes in ploidy.

The National Toxicology Program 13-week study of TCE gavage exposure in 10 F344/N rats (125 to 2,000 mg/kg [males] and 62.5 to 1,000 mg/kg [females]) and in B6C3F1mice (375 to 6,000 mg/kg) reported all rats survived the 13-week study, but males receiving 2,000 mg/kg exhibited a 24% difference in final body weight. The study descriptions of pathology in rats and mice were not very detailed and included only mean liver weights. The rats had increased pulmonary vasculitis at the highest concentration of TCE and that viral titers were positive for Sendai virus and no liver effects were noted for them in the study. For mice, liver weights (both absolute and percent liver/body weight) were reported to increase in a dose-related fashion with TCE –exposure and to be increased by more than 10% in 750 mg/kg TCE-exposed males and 1,500 mg/kg or more TCE-exposed females. Hepatotoxicity was reported as centrilobular necrosis in 6/10 males and 1/10 females exposed to 6,000 mg/kg TCE and multifocal areas of calcifications scattered throughout 3,000 mg/kg TCE exposed male mice and only a single female 6,000 mg/kg dose, considered to be evidence of earlier hepatocellular necrosis. One female mouse exposed to 3,000 mg/kg TCE also had a hepatocellular adenoma, an extremely rare lesion in female mice of this age (20 weeks). However, at the lowest dose of exposure, was a consistent decrease in liver weigh in female and male mice after 13 weeks of TCE exposure.

Kawamoto et al. (1988) exposed rats to 2 g/kg TCE subcutaneously for 15 weeks and reported TCE-induced increases in liver weight. They also reported increase in cytochrome P450, cytochrome b-5, and NADPH cytochrome c reductase. The difficulties in relating this route of exposure to more environmentally relevant ones is discussed in Section E.2.2.11.

For 2-year or lifetime studies of TCE exposure a consistent hepatocarcinogenic response has been observed in mice of differing strains and genders and from differing routes of exposure. However, for rat studies some studies have been confounded by mortality from gavage error or the toxicity of the dose of TCE administered. In some studies, a relative insensitive strain of rat has been used. However, in general it appears that the mouse is more sensitive than the rat to TCE-induced liver cancer. Three studies give results the authors consider to be negative for TCE-induced liver cancer in mice, but have either design and/or reporting limitations, or are in strains and paradigms with apparent low ability for liver cancer induction or detection.

Fukuda et al. (1983) reported a 104-week inhalation bioassay in female Crj:CD-1 (ICR) mice and female Crj:CD (S-D) rats exposed to 0, 50, 150 and 450 ppm TCE ($n = 50$). There were no reported incidences of mice or rats with liver tumors for controls indicative of relatively insensitive strains used in the study for liver effects. While TCE was reported to induce a number of other tumors in mice and rats in this study, the incidence of liver tumors was less than 2% after TCE exposure. Of note is the report of cystic cholangioma reported in 1 group of rats.

Henschler et al. (1980) exposed NMRI mice and WIST random bred rats to 0, 100, and 500 ppm TCE for 18 months ($n = 30$). This study is limited by short duration of exposure, low number of animals, and low survival in rats. Control male mice were reported to have one hepatocellular carcinoma and 1 hepatocellular adenoma with the incidence rate unknown. In the 100 ppm TCE exposed group, 2 hepatocellular adenomas and 1 mesenchymal liver tumor were reported. No liver tumors were reported at any dose of TCE in female mice or controls. For male rats, only 1 hepatocellular adenomas at 100 ppm was reported. For female rats no liver tumors were reported in controls, but 1 adenoma and 1 cholangiocarcinoma was reported at 100 ppm TCE and at 500 ppm TCE, 2 cholangioadenomas, a relatively rare biliary tumor, was reported. The difference in survival in mice, did not affect the power to detect a response, as was the case for rats. However, the low number of animals studied, abbreviated exposure duration, and apparently low sensitivity of this paradigm (i.e., no background response in controls) suggests a study of limited ability to detect a TCE carcinogenic liver response. Of note is that both Fukuda et al. (1983) and Henschler et al. (1980) report rare biliary cell derived tumors in rats in relatively insensitive assays.

Van Duuren et al. (1979), exposed mice to 0.5 mg/mouse to TCE via gavage once a week in 0.1 mL trioctanion ($n = 30$). Inadequate design and reporting of this study limit that ability to use the results as an indicator of TCE carcinogenicity.

The NCI (1976) study of TCE was initiated in 1972 and involved the exposure of Osborn-Mendel rats and B6C3F1 mice to varying concentrations of TCE. The animals were coexposed to a number of other carcinogens as exhalation as multiples studies and control animals all shared the same laboratory space. Treatment duration was 78 weeks and animals received TCE via gavage in corn oil at 2 doses ($n = 20$ for controls, but $n = 50$ for treatment groups). For rats, the high dose was reported to result in significant mortality (i.e., 47/50 high-dose rats died before scheduled termination of the study). A low incidence of liver tumors was reported for controls and carbon tetrachloride positive controls in rats from this study. In B6C3F1 mice, TCE was reported to increase incidence of hepatocellular carcinomas in both doses and both genders of mice (~1,170 and 2,340 mg/kg for males and 870 and 1,740 mg/kg for female mice). Hepatocellular carcinoma diagnosis was based on histologic appearance and metastasis to the lung. The tumors were described in detail and to be heterogeneous "as described in the literature" and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure.

For male rats, noncancer pathology in the NCI (1976) study was reported to include increased fatty metamorphosis after TCE exposure and angiectasis or abnormally enlarged blood vessels. Angiectasis can be manifested by hyperproliferation of endothelial cells and dilatation of sinusoidal spaces. The authors conclude that due to mortality, "the test is inconclusive in rats." They note the insensitivity of the rat strain used to the positive control of carbon tetrachloride exposure.

The NTP (1990) study of TCE exposure in male and female F344/N rats, and B6C3F1 mice (500 and 1,000 mg/kg for rats and 1,000 mg/kg for mice) is limited in the ability to demonstrate a dose-response for hepatocarcinogenicity. There was also little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study. However, by the end of a 2-year cancer bioassay, liver tumor induction can be a significant factor in any changes in liver weight. No treatment-related increase in necrosis in the liver was observed in mice. A slight increase in the incidence of focal necrosis was noted for TCE-exposed male mice (8 vs. 2% in control) with a slight reduction in fatty metamorphosis in treated male mice (0 treated vs. 2 control animals) and in female mice a slight increase in focal inflammation (29 vs. 19% of animals) and no other changes. Therefore, this study did not show concurrent evidence of liver toxicity but did show TCE-induced neoplasia after 2 years of TCE exposure in mice. The administration of TCE was reported to cause earlier expression of tumors as the first animals with carcinomas were 57 weeks for TCE-exposed animals and 75 weeks for control male mice.

The NTP (1990) study reported that TCE exposure was associated with increased incidence of hepatocellular carcinoma (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. Hepatocellular carcinomas had markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies and variations in nuclear appearance. Furthermore, in many instances several or all of the abnormalities were present in different areas of the tumor and variations in architecture with some of the hepatocellular carcinomas having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors.

For rats, the NTP (1990) study reported no treatment-related non-neoplastic liver lesions in males and a decrease in basophilic cytological change reported from TCE-exposure in female rats. The results for detecting a carcinogenic response in rats were considered to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error.

The NTP (1988) study of TCE exposure in four strains of rats to “diisopropylamine-stabilized TCE” was also considered inadequate for either comparing or assessing TCE-induced carcinogenesis in these strains of rats because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data. TCE gavage exposures of 0, 500 or 1,000 mg/kg per day (5 days per week, for 103 weeks) male and female rats was also marked by a large number of accidental deaths (e.g., for high-dose male Marshal rats 25 animals were accidentally killed). Results from a 13-week study were briefly mentioned in the report and indicated exposure levels of 62.5−2,000 mg/kg TCE were not associated with decreased survival (with the exception of 3 male August rats receiving 2,000 mg/kg TCE) and that the administration of the chemical for 13 weeks was not associated with histopathological changes. In regard to evidence of liver toxicity, the 2-year study of TCE exposure reported no evidence of TCE-induced liver toxicity described as non-neoplastic changes ACI, August, Marshal, and Osborne-Mendel rats. Interestingly, for the control animals of these four strains there was, in general, a low background level of focal necrosis in the liver of both genders. In summary, the negative results in this bioassay are confounded by the killing of a large portion of the animals accidently by experimental error but TCE-induced overt liver toxicity was not reported.

Maltoni et al. (1986) reported the results of several studies of TCE via inhalation and gavage in mice and rats. A large number of animals were used in the treatment groups but the focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype given and limited reporting of non-neoplastic changes in the liver. Accidental death by gavage error was reported not to occur in this study. In regards to effects of TCE exposure on survival, “a nonsignificant excess in mortality” correlated to TCE treatment was observed only in female rats (treated by ingestion with the compound) and in male B6C3F1 mice. TCE-induced effects on body weight were reported to be absent in mice except for one experiment (BT 306 bis) in which a slight nondose correlated decrease was found in exposed animals. “Hepatoma” was the term used to describe all malignant tumors of hepatic cells, of different subhistotypes, and of various degrees of malignancy and were reported to be unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype tumors were described as usual type observed in Swiss and B6C3F1 mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. Swiss mice from this laboratory were reported to have a low incidence of hepatomas without treatment (1%). The relatively larger number of animals used in this bioassay (n = 90 to 100), in comparison to NTP standard assays, allows for a greater power to detect a response.

TCE exposure for 8 weeks via inhalation at 100 ppm or 600 ppm may have been associated with a small increase in liver tumors in male mice in comparison to concurrent controls during the life span of the animals. In Swiss mice exposed to TCE via inhalation for 78 weeks there a reported increase in hepatomas associated with TCE treatment that was dose-related in male but not female Swiss mice. In B6C3F1 mice exposed via inhalation to TCE for 78 weeks, the results from one experiment indicated a greater increase in liver cancer in females than male mice but in a second experiment in males there was a TCE-exposure associated increase in hepatomas. Although the mice were supposed to be of the same strain, the background level of liver cancer was significantly different in male mice. The finding of differences in response in animals of the same strain but from differing sources has also been reported in other studies for other endpoints (see Section E.3.1.2). However, for both groups of male B6C3F1 mice the background rate of liver tumors over the lifetime of the mice was less than 20%.

For rats, there were 4 liver angiosarcomas reported (1 in a control male rat, 1 both in a TCE-exposed male and female at 600 ppm TCE for 8 weeks, and 1 in a female rat exposed to 600 ppm TCE for 104 weeks) but the specific results for incidences of hepatocellular "hepatomas" in treated and control rats were not given. Although the Maltoni et al. (1986) concluded that the small number was not treatment-related, the findings were brought forward because of the extreme rarity of this tumor in control S-D rats, untreated or treated with vehicle materials. In rats treated for 104 weeks, there was no report of a TCE treatment-related increase in liver cancer in rats. This study only presented data for positive findings so it did not give the background or treatment-related findings in rats for liver tumors in this study. Thus, the extent of background tumors and sensitivity for this endpoint cannot be determined. Of note is that the S-D strain used in this study was also noted in the Fukuda et al. (1983) study to be relatively insensitive for spontaneous liver cancer and to also be negative for TCE-induced hepatocellular liver cancer induction in rats. However, like Fukuda et al. (1983) and Henschler et al. (1980), that reported rare biliary tumors in insensitive strains of rat for hepatocellular tumors, Maltoni et al. (1986) reported a relatively rare tumor type, angiosarcoma, after TCE exposure in a relatively insensitive strain for "hepatomas." As noted above, many of the rat studies were limited by premature mortality due to gavage error or premature mortality (Henschler et al., 1980; NCI, 1976; NTP, 1990, 1988), which was reported not occur in Maltoni et al. (1986).

There were other reports of TCE carcinogenicity in mice from chronic exposures that were focused primarily on detection of liver tumors with limited reporting of tumor phenotype or non-neoplastic pathology. Herren-Freund et al. (1987) reported that male B6C3 F1 mice given 40 mg/L TCE in drinking water had increased tumor response after 61 weeks of exposure. However, concentrations of TCE fell by about ½ at this dose of TCE during the twice a week change in drinking water solution so the actual dose of TCE the animals received was less than 40 mg/L. The percent liver/body weight was reported to be similar for control and TCE-exposed mice at the end of treatment. However, despite difficulties in establishing accurately the dose received, an increase in adenomas per animal and an increase in the number of animals with hepatocellular carcinomas were reported to be associated with TCE exposure after 61 weeks of exposure and without apparent hepatomegaly. Anna et al. (1994) reported tumor incidences for male B6C3F1 mice receiving 800 mg/kg/d TCE via gavage (5 days/week for

76 weeks). All TCE-treated mice were reported to be alive after 76 weeks of treatment. Although the control group contained a mixture of exposure durations (76−134 weeks) and concurrent controls had a very small number of animals, TCE-treatment appeared to increase the number of animals with adenomas, the mean number of adenomas and carcinomas, but with no concurrent TCE-induced cytotoxicity.

E.2.4.4. *Summary of Results For Subchronic and Chronic Effects of Dichloroacetic Acid (DCA) and Trichloroacetic Acid (TCA): Comparisons With Trichloroethylene (TCE)*

There are no similar studies for TCA and DCA conduced at 6 weeks and with the range of concentrations examined in Buben and O'Flaherty (1985) for TCE. In general, many studies of DCA and TCA have been conducted at few and high concentrations, with shortened durations of exposure, and varying and low numbers of animals to examine primarily a liver tumor response in mice. However, the analyses presented in Section E.2.4.2 gives comparisons of administered TCA and DCA dose-responses for liver weight increases for a number of studies in combination as well as comparing such dose-responses to that of TCE and its oxidative metabolism. As stated above, many subchronic studies of DCA and TCA have focused on elucidating a relationship between dose and hypothesized events that may be indicators of carcinogenic potential that have been described in chronic studies with a focus on indicators of peroxisome proliferation and DNA synthesis. Many chronic studies have focused on the nature of the DCA and TCA carcinogenic response in mouse liver through examination of the tumors induced.

Most all of the chronic studies for DCA and TCA have been carried out in mice. As the database for examination of the ability of TCE to induce liver tumors in rats includes several studies that have been limited in ability determine a carcinogenic response in the liver, the database for DCA and TCA in rats is even more limited. For TCA, the only available study in rats (DeAngelo et al., 1997) has been frequently cited in the literature to indicate a lack of response in this species for TCA-induced liver tumors. Although reporting an apparent dose-related increase in multiplicity of adenomas and an increase in carcinomas over control at the highest dose, DeAngelo et al. (1997) use such a low number of animals per treatment group (n = 20−24) that the ability of this study to determine a statistically significant increase in tumor response and to be able to determine that there was no treatment-related effect are limited. A power calculation of the study shows that the type II error, which should be >50%, was less than 8% probability for incidence and multiplicity of all tumors at all exposure DCA concentrations with the exception of the incidence of adenomas and adenomas and carcinomas for 0.5 g/L treatment group (58%) in which there was an increased in adenomas reported over control

(15 vs. 4%) that was the same for adenomas and carcinomas combined. Therefore, the designed experiment could accept a false null hypothesis and erroneously conclude that there is no response due to TCA treatment. Thus, while suggesting a lower response than for mice for liver tumor induction, it is inconclusive for determination of whether TCA induces a carcinogenic response in the liver of rats.

For DCA, there are two reported long-term studies in rats (DeAngelo et al., 1996; Richmond et al., 1995) that appear to have reported the majority of their results from the same data set and which consequently were subject to similar design limitations and DCA-induced neurotoxicity in this species. DeAngelo et al. (1996) reported increased hepatocellular adenomas and carcinomas in male F344 rats exposed for 2 years. However, the data from exposure concentrations at a 5 g/L dose had to be discarded and the 2.5 g/L DCA dose had to be continuously lowered during the study due to neurotoxicity. There was a DCA-induced increased in adenomas and carcinomas combined reported for the 0.5 g/L DCA (24.1 vs. 4.4% adenomas and carcinomas combined in treated vs. controls) and an increase at a variable dose started at 2.5 g/L DCA and continuously lowered (28.6 vs. 3.0% adenomas and carcinomas combined in treated vs. controls). Only combined incidences of adenomas and carcinomas for the 0.5 g/L DCA exposure group was reported to be statistically significant by the authors although the incidence of adenomas was 17.2 versus 4% in treated versus control rats. Hepatocellular tumor multiplicity was reported to be increased in the 0.5 g/L DCA group (0.31 adenomas and carcinomas/animal in treated vs. 0.04 in control rats) but was reported by the authors to not be statistically significant. At the starting dose of 2.5 g/L that was continuously lowered due to neurotoxicity, the increased multiplicity of hepatocellular carcinomas was reported by the authors to be to be statistically significant (0.25 carcinomas/animals vs. 0.03 in control) as well as the multiplicity of combined adenomas and carcinomas (0.36 adenomas and carcinomas/animals vs. 0.03 in control rats). Issues that affect the ability to determine the nature of the dose-response for this study include (1) the use of a small number of animals (n = 23, n =21 and n = 23 at final sacrifice for the 2.0 g/L NaCl control, 0.05 and 0.5 g/L treatment groups) that limit the power of the study to both determine statistically significant responses and to determine that there are not treatment-related effects (i.e., power) (2) apparent addition of animals for tumor analysis not present at final sacrifice (i.e., 0.05 and 0.5 g/L treatment groups), and (3) most of all, the lack of a consistent dose for the 2.5 g/L DCA exposed animals. Similar issues are present for the study of Richmond et al. (1995) which was conducted by the same authors as DeAngelo et al. (1996) and appeared to be the same data set. The Richmond et al. (1995) data for the 2 g/L NaCl. 0.05 g/L DCA and 0.5 g/L DCA exposure groups were the same data set reported by DeAngelo et al. (1996) for these groups. Additional data was reported for F344 rats administered and 2.5 g/L DCA that, due to hind-limb paralysis, were sacrificed 60 weeks (DeAngelo et al., 1996). Tumor multiplicity was not reported by the authors. There was a small difference in reports of the results between the two studies for the same data for the 0.5 g/L DCA group in which Richmond et al. (1995) reported a 21% incidence of adenomas and DeAngelo et al. (1996) reported a 17.2% incidence. The authors did not report any of the results of DCA-induced increases of adenomas and carcinomas to be statistically significant. The same issues discussed above for DeAngelo et al. (1996) apply to this study. Similar to the DeAngelo study of TCA in rats (DeAngelo et al., 1997) the study of DCA exposure in rats reported by DeAngelo et al. (1996) and Richmond et al. (1995), the use of small numbers of rats limits the detection of treatment-related effects and the ability to determine whether there was no treatment related effects (Type II error), especially at the low concentrations of DCA exposure.

For mice the data for both DCA and TCA is much more extensive and has shown that both DCA and TCA induced liver tumors in mice. Many of the studies are for relatively high concentrations of DCA or TCA, have been conducted for a year or less, and have focused on the nature of tumors induced to ascertain potential MOAs and to make inferences as to whether TCE-induced tumors in mice are similar. As shown previously in Section E.2.4.2, the dose-response curves for increased liver weight for TCE administration in male mice are more similar to those for DCA administration and TCE oxidative metabolism than for direct TCA administration. There are two studies in male B6C3F1 mice that attempt to examine multiple concentrations of DCA and TCA for 2-year studies (DeAngelo et al., 1999, 2008) at doses that do not induce cytotoxicity and attempt to relate them to subchronic changes and peroxisomal enzyme induction. However, the DeAngelo et al. (2008) study was carried out in B6C3F1 mice that were of large size and prone to liver cancer and premature mortality limiting its use for the determination of TCA-dose response in a 2-year bioassay. One study in female B6C3F1 mice describes the dose-response for liver tumor induction at a range of DCA and TCA concentrations after 51 or 82 weeks (Pereira, 1996) with a focus on the type of tumor each compound produced.

DeAngelo et al. (1999) conducted a study of DCA exposure to determine a dose response for the hepatocarcinogenicity of DCA in male B6C3F1 mice over a lifetime exposure and especially at concentrations that did not illicit cytotoxicity or were for abbreviated exposure durations. DeAngelo et al. (1999) used 0.05, 0.5, 1.0, 2.0, and 3.5 g/L exposure concentrations of DCA in their 100-week drinking water study. The number of animals at final sacrifice was generally low in the DCA treatment groups and variable (i.e., $n = 50$, $n = 33$, $n = 24$, $n = 32$, $n = 14$, and $n = 8$ for control, 0.05, 0.5, 1, 2.0, and 3.5 g/L DCA exposure groups). It was apparent that animals that died unscheduled deaths between weeks 79 and 100 were included in data reported for 100 weeks. Although the authors did not report how many animals were included in the 100-week results, it appeared that the number was no greater than 1 for the control, 0.05, and 0.5 exposure groups and varied between 3 and 7 for the higher DCA exposure groups. The multiplicity or number of hepatocellular carcinomas/animals was reported to be significantly increased over controls in a dose-related manner at all DCA treatments including 0.05 g/L DCA, and a NOEL reported not to be observed by the authors (i.e., 0.28, 0.58, 0.68, 1.29, 2.47, and 2.90 hepatocellular carcinomas/animal for control, 0.05, 0.5, 1.0, 2.0, and 3.5 g/L DCA). Between the 0.5 and 3.5 g/L exposure concentrations of DCA the magnitude of increase in multiplicity was similar to the increases in magnitude in dose. The incidence of hepatocellular carcinomas were reported to be increased at all doses as well but not reported to be statistically significant at the 0.05 g/L exposure concentration. However, given that the number of mice examined for this response ($n = 33$), the power of the experiment at this dose was only 16.9% to be able to determine that there was not a treatment related effect. The authors did not report the incidence or multiplicity of adenomas for the 0.05 g/L exposure group in the study and neither did they report the incidence or multiplicity of adenomas and carcinomas in combination. For the animals surviving from 79 to 100 weeks of exposure, the incidence and multiplicity of adenomas peaked at 1 g/L while hepatocellular carcinomas continued to increase at the higher doses. This would be expected where some portion of the adenomas would either regress or progress to carcinomas at the higher doses.

DeAngelo et al. (1999) reported that peroxisome proliferation was significantly increased at 3.5 g/L DCA only at 26 weeks, not correlated with tumor response, and to not be increased at either 0.05 or 0.5 g/L treatments. The authors concluded that DCA-induced carcinogenesis was not dependent on peroxisome proliferation or chemically sustained proliferation, as measured by DNA synthesis. DeAngelo et al. (1999) reported not only a dose-related increase in DCA-induced liver tumors but also a decrease in time-to-tumor associated with DCA exposure at the lowest levels examined. In regards to cytotoxicity there appeared to be a treatment but not dose-related increase in hepatocellular necrosis that did not involve most of the liver from 1 to 3.5 g/L DCA exposures for 26 weeks of exposure that decreased by 52 weeks with no necrosis observed at the 0.5 g/L DCA treatment for any exposure period.

Hepatomegaly was reported to be absent by 100 weeks of exposure at the 0.05 and 0.5 g/L exposures while there was an increase in tumor burden reported. However, slight hepatomegaly was present by 26 weeks in the 0.5 g/L group and decreased with time. Not only did the increase in multiplicity of hepatocellular carcinomas increase proportionally with DCA exposure concentration after 79−100 weeks of exposure, but so did the increases in percent liver/body weight. DeAngelo et al. (1999) presented a figure comparing the number of hepatocellular carcinomas/animal at 100 weeks compared with the percent liver/body weight at 26 weeks that showed a linear correlation (r^2 = 0.9977) while peroxisome proliferation and DNA synthesis did not correlate with tumor induction profiles. The proportional increase in liver weight with DCA exposure was also reported for shorter durations of exposure as noted in Section E.2.4.2. The findings of the study illustrates the importance of examining multiple exposure levels at lower concentrations, at longer durations of exposure and with an adequate number of animals to determine the nature of a carcinogenic response. Although Carter et al. (1995) suggested that there is evidence of DCA-induced cytotoxicity (e.g., loss of cell membranes and apparent apoptosis) at higher levels, the 0.5 g/L exposure concentration has been shown by DeAngelo et al. (1999) to increase hepatocellular tumors after 100 weeks of treatment without concurrent peroxisome proliferation or cytotoxicity in mice.

As noted in detail in E. 2.3.2.13, DeAngelo et al. (2008) exposed male B6C3F1 mice to neutralized TCA in drinking water to male B6C3 F1 mice in three studies. Rather than using 5 exposure levels that were generally 2-fold apart, as was done in DeAngelo et al. (1999) for DCA, DeAngelo et al. (2008) studied only 3 doses of TCA that were an order of magnitude apart which limits the elucidation of the shape of the dose-response curve. In addition DeAngelo et al. (2008) contained 2 studies, each conducted in a separate laboratories, for the 104-week data so that the two lower doses were studied in one study and the highest dose in another. The first study was conducted using 2 g/L NaCl, or 0.05, 0.5, or 5 g/L TCA in drinking water for 60 weeks (Study #1) while the other two were conducted for a period of 104 weeks (Study #2 with 2.5 g/L neutralized acetic acid or 4.5 g/L TCA exposure groups and Study #3 with deionized water, 0.05 and 0.5 g/L TCA exposure groups). In the studies reported in DeAngelo et al. (2008) a small number of animals has been used for the determination of a tumor response (~n = 30 at final necropsy), but for the data for liver weight or PCO activity at interim sacrifices the number was even smaller (n = 5). The percent liver/body weight changes at 4 weeks in Study #1 have been included in the analysis for all TCA data in Section E.2.4.2, and are consistent with that data. Although there was a 10-fold difference in TCA exposure concentration, there was a 9, 16, and 35% increase in liver weight over control for the 0.05, 0.5, and 5 g/L TCA exposures. PCO activity varied 2.7-fold as baseline controls but the increase in PCO activity at 4 weeks was 1.3-, 2.4-, and 5.3-fold of control for the 0.05, 0.5, and 5 g/L TCA exposure groups in Study #1. The incidence data for adenomas observed at 60 weeks was 2.1-, 3.0-, and 5.4-fold of control values and the fold increases in multiplicity were similar after 0.05, 0.5, and 5.0 g/L TCA. Thus, in general the dose-response for TCA-induced liver weight increases at 4 weeks was similar to the magnitude of induction of adenomas at 60 weeks. Such a result is more consistent with the ability of TCA to induce tumors and increases in liver weight at low doses with little change with increasing dose as shown by this study and the combined data for TCA liver weight induction by administered TCA presented in Section E.2.4.2.

While the 104-week data from Study's #2 and #3 could have been more valuable for determination of the dose-response as it would have allowed enough time for full tumor expression, serious issues are apparent for Study #3, which was reported to have a 64% incidence rate of adenomas and carcinomas for controls while that of Study #2 was 12%. As stated in Section E.2.3.2.13, the mice in Study #3 were of larger size than those of either Study #1 or #2 and the large background rate of tumors reported is consistent with mice of these size (Leakey et al., 2003b). However, the large background rate and increased mortality for these mice limit their use for determining the nature of the dose-response for TCA liver carcinogenicity. Examination of the data for treatment groups shows that there was no difference in any of the results between the 0.5 g/L (Study #3) and 5 g/L (Study #2) TCA exposure groups (i.e., adenoma, carcinoma, and combinations of adenoma and carcinoma incidence and multiplicity) for 104 weeks of exposure. For these same exposure groups, but at 60 weeks of exposure (Study #1), there was a 2-fold increase in multiplicity for adenomas, and for adenomas and carcinomas combined between the 0.5 and 5.0 g/L TCA exposure groups. At the two lowest doses of 0.05 and 0.5 g/L TCA from Study #3 in the large tumor prone mice, the differences in the incidences and multiplicities for all tumors were 2-fold at 104 weeks. These results are consistent with (1) the two highest exposure levels reaching a plateau of response after a long enough duration of exposure for full expression of the tumors (i.e., ~90% of animals having liver tumors at the 0.5 and 5 g/L exposures) with the additional tumors observed in a tumor-prone paradigm. Thus, without use of the 0.05 and 0.5 g/L TCA data from Study #3, only the 4.5 g/L TCA data from Study #2 can be used for determination of the TCA cancer response in a 2-year bioassay.

To put the 64% incidence data for carcinomas and adenomas reported in DeAngelo et al. (2008) for the control group of Study #3 in context, other studies cited in this review for male B6C3F1 mice show a much lower incidence in liver tumors with: (1) NCI (1976) study of TCE reporting a colony control level of 6.5% for vehicle and 7.1% incidence of hepatocellular carcinomas for untreated male B6C3F1 mice (n = 70−77) at 78 weeks, (2) Herren-Freund et al. (1987) reporting a 9% incidence of adenomas in control male B6C3F1 mice with a multiplicity of 0.09 ± 0.06 and no carcinomas (n = 22) at 61 weeks, (3) NTP (1990) reporting an incidence of 14.6% adenomas and 16.6% carcinomas in male B6C3F1 mice after 103 weeks (n = 48), and (4) Maltoni et al. (1986) reporting that B6C3F1 male mice from the "NCI source" had a 1.1% incidence of "hepatoma" (carcinomas and adenomas) and those from "Charles River Co." had a

18.9% incidence of "hepatoma" during the entire lifetime of the mice (*n* = 90 per group). The importance of examining an adequate number of control or treated animals before confidence can be placed in those results in illustrated by Anna et al. (1994) in which at 76 weeks 3/10 control male B6C3F1 mice that were untreated and 2/10 control animals given corn oil were reported to have adenomas but from 76 to 134 weeks, 4/32 mice were reported to have adenomas (multiplicity of 0.13 ± 0.06) and 4/32 mice were reported to have carcinomas (multiplicity of 0.12 ± 0.06). Thus, the reported combined incidence of carcinomas and adenomas of 64% reported by DeAngelo et al. (2008) for the control mice of Study #3, not only is inconsistent and much higher than those reported in Studies #1 and #2, but also much higher than reported in a number of other studies of TCE.

Trying to determine a correspondence with either liver weight increases or increases in PCO activity after shorter periods of exposure will be depend whether data reported in Study #3 in the 104 week studies can be used. DeAngelo et al. (2008) report a regression analyses that compare "percent of hepatocellular neoplasia," indicated by tumor multiplicity, with TCA dose, represented by estimations of the TCA dose in mg/kg/d, and with PCO activity for the 60-week and 104-week data. Whether adenomas and carcinomas combined or individual tumor type were used in these analysis was not reported by the authors. Concerns arise also from comparing PCO activity at the end of the experiments, when there was already a significant tumor response, rather than at earlier time points. Such PCO data may not be useful as an indicator key event in tumorigenesis when tumors are already present. In addition regression analyses of these data are difficult to interpret because of the dose spacing of these experiments as the control and 5 g/L exposure levels will basically determine the shape of the dose-response curve. The 0.05 and 0.5 g/L exposure levels are close to the control value in comparison to the 5 g/L exposure level, the dose response appears to be linear between control and the 5.0 g/L value with the two lowest doses not affectly changing the slope of the line (i.e., "leveraging" the regression). Thus, the value of these analyses is limited by (1) use of data from Study #3 in a tumor prone mouse that is not comparable to those used in Studies #1 and #2, (2) the appropriateness of using PCO values from later time points and the variability in PCO control values (3) the uncertainty of the effects of palatability on the 5 g/L TCA results which were reported in one study to reduce drinking water consumption, and (4) the dose-spacing of the experiment.

DeAngelo et al. (2008) attempt to identify a NOEL for tumorigenicity using tumor multiplicity data and estimated TCA dose. However, it is not an appropriate descriptor for these data, especially given that "statistical significance" of the tumor response is the determinant used by the authors to support the conclusions regarding a dose in which there is no TCA-induced effect. Due to issues related to the appropriateness of use of the concurrent control in Study #3, only the 60-week experiment (i.e., Study #1) is useful for the determination of tumor dose-response. Not only is there not allowance for full expression of a tumor response at the 60-week time point but a power calculation of the 60-week study shows that the type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was 41 and 71% for incidence and 7 and 15% for multiplicity of adenomas for the 0.05 and 0.5 g/L TCA exposure groups. For the combination of adenomas and carcinomas, the power calculation was 8 and 92% for incidence and 6 and 56% for multiplicity at 0.05 and 0.5 g/L TCA exposure. Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment.

Pereira (1996) examined the tumor induction in female B6C3 F1 mice and demonstrate that foci, adenoma, and carcinoma development in mice are dependent on duration of exposure, or period of observation in the case of controls, for full expression of a carcinogenic response. In control female mice a 360- versus 576-day observation period showed that at 360 days no foci or carcinomas and only 2.5% of animals had adenomas whereas by 576 days of observation, 11% had foci, 2% adenomas, and 2% had carcinomas. For DCA and TCA treatments, foci, adenomas, and carcinoma incidence and multiplicity did not reach full expression until 82 weeks at the 3 doses employed (2.58 g/L DCA, 0.86 g/L DCA, 0.26 g/L DCA, 3.27 g/L TCA, 1.1.0 g/L TCA, and 0.33 g/L TCA). Although the numbers of animals were relatively low and variable at the two highest doses (18−28 mice) there were 50−53 mice studied at the lowest dose level and 90 animals studied in the control group. The results of Pereira (1996) show that not only were the incidence of mice with foci, adenoma, and carcinomas greatly increased with duration of exposure, but that concentration also affected the nature and magnitude of the response in female mice. At 2.86 g/L, 0.86 g/L, 0.26 g/L DCA exposures and controls, after 82 weeks the incidence of adenomas in female B6C3 F1 mice was reported to be 84.2, 25.0, 6.0, and 2.2%, respectively, and carcinomas to be 26.3, 3.6, 0, and 2.2%, respectively. For the multiplicity or number of tumors/animal at these same exposure levels of DCA, the multiplicity was reported to be 5.58, 0.32, 0.06, and 0.02 adenomas/animal, and 0.37, 0.04, 0, and 0.02 carcinomas/animal. Thus, for DCA exposure in female mice, for ~3-fold increases in DCA exposure concentration, after 82 weeks of exposure there was a similar magnitude of increase in adenomas incidence with much greater increases in multiplicity. For hepatocellular carcinoma induction, there was no increase in the incidence or multiplicity or carcinomas between the control and 0.33 g/L DCA dose. At 3.27, 1.10, and 0.33 g/L TCA and controls, after 82 weeks the incidence of adenomas in female B6C3F1 mice was reported to be 38.9, 11.1, 7.6, and 2.2%, respectively, and carcinomas to be 27.8, 18.5, 0, and 2.2%, respectively. At these same exposure levels of TCA, the multiplicity was reported to be 0.61, 0.11, 0.08, and 0.02 adenomas/animal, and 0.39, 0.22, 0, and 0.02 carcinomas/animal, respectively. Thus, for TCA, the incidences of adenomas were lower at the two highest doses than DCA and the ~3-fold differences in dose between the two lowest doses only resulted in ~50% increase in incidences of adenomas. For incidence of carcinomas the ~3-fold difference in dose between the two highest doses only resulted in ~50% increase in carcinoma incidence. A similar pattern was reported for multiplicity after TCA exposure. Foci were also examined and, in general., were similar to adenomas regarding incidence and multiplicity. Thus, the dose-response curve for tumor induction in female mice differed between DCA and TCA after 82 weeks of exposure with TCA having a much less steep dose-response curve than DCA. This is consistent with the pattern of liver weight increases reported for male B6C3F1 mice in Section E.2.4.2.

DeAngelo et al. (1999) report a linear increase in incidence and multiplicity of hepatocellular carcinomas that is proportional to dose and as well as proportional to the magnitude of liver weight increase from subchronic exposure to DCA. However, the studies of DeAngelo et al. (2008) and Pereira (1996) are suggestive that TCA induced increase in tumor incidence are less proportional to increases in dose as are liver weight increases from subchronic exposure. Given that TCE subchronic exposure also induced an increase in liver weight that was proportional to dose (i.e., similar to DCA but not TCA), it is of interest as to whether the dose-response for TCE induced liver cancer in mice was similar. The database for TCE, while consistently showing a induction of liver tumors in mice, is very limited for making inferences regarding the shape of the dose-response curve. For many of these experiments multiplicity was not given only liver tumor incidence. NTP (1990), Bull et al. (2002), Anna et al. (1994) conducted gavage experiments in which they only tested one dose of ~1,000 mg/kg/d TCE. NCI (1976) tested 2 doses that were adjusted during exposure to an average of 1,169 mg/kg/d and 2,339 mg/kg/d in male mice with only 2-fold dose spacing in only 2 doses tested. Maltoni et al. (1988) conducted inhalation experiments in 2 sets of B6C3F1 mice and one set of Swiss mice at 3 exposure concentrations that were 3-fold apart in magnitude between the low and mid-dose and 2-fold apart in magnitude between the mid- and high-dose. However, for one experiment in male B6C3F1 mice, the mice fought and suffered premature mortality and for two the experiments in B6C3F1 mice, although using the same strain, the mice were obtained from differing sources with very different background liver tumor levels. For the Maltoni et al. (1988) study a general descriptor of "hepatoma" was used for liver neoplasia rather than describing hepatocellular adenomas and carcinomas so that comparison of that data with those from other experiments is difficult. More importantly, while the number of adenomas and carcinomas may be the same between treatments or durations of exposure, the number of adenomas may decrease as the number of carcinomas increase during the course of tumor progression. Such information is lost by using only a hepatoma descriptor. Maltoni et al. (1988) did not report an increase over control for 100 ppm TCE for the Swiss group and one of the B6C3F1 groups and only a slight increase (1.12-fold) in the second B6C3F1 group. At 300 ppm TCE exposure, the incidences of hepatoma were 2-fold of control values for the Swiss, 4-fold of control for group of B6C3F1 mice, and 1.6-fold of control for the other group of B6C3F1 mice. At 600 ppm TCE the incidences of hepatoma were 3.3-fold of control for the Swiss group, 6.1-fold of control for one group of B6C3F1 mice, and 1.2-fold for the other group of B6C3F1 mice. Thus, for each group of TCE exposed mice in the Maltoni et al. (1988) inhalation study, the background levels of hepatomas and the shape of the dose-response curve for TCE-hepatoma induction were variable. However, an average of the increases, in terms of fold of control, between the 3 experiments gives a ~2.9-fold increase between the low- and mid-dose (100 ppm and 300 ppm) and ~1.4-fold increase between the mid- and high-dose (300 ppm and 600 pm) groups. Although such a comparison obviously has a high degree of uncertainty associated with it, it suggests that the magnitude of TCE-induced hepatoma increases over control is similar to the 3- and 2-fold difference in the magnitude of exposure concentrations between these doses. Therefore, the increase in TCE-induced liver tumors would roughly proportional to the magnitude of exposure dose. This result would be similar to the result for the concordance of the increases in liver weight and exposure concentration observed 28−42 day exposures to TCE (see Section E.2.4.2) using oral data from B6C3F1 and Swiss mice, and inhalation data from NMRI mice. The available inhalation data for TCE induced liver weight dose-response is from one study in a strain derived from Swiss mice (Kjellstrand et al., 1983b) and was conducted in male and female mice with comparable doses of 75 ppm and 300 ppm TCE. However, male mice of this strain exhibited decreased body weight at the 300 ppm level, which can affect percent liver/body weight increases. The magnitude of TCE-induced increases in liver weight between the 75 ppm and 300 ppm exposures were ~1.80-fold for males (1.50 vs. 1.90-fold of control liver weights) and 4.2-fold for females (1.27- vs. 2.14-fold of control liver weight) in this strain. Female mice were examined in one study each of Swiss and B6C3F1 mice by Maltoni et al. (1988). Both the Swiss and B6C3F1 studies reported increases in incidences of hepatomas over controls only at the 600 ppm TCE level in female mice indicating less of a response than males. Similarly, the Kjellstrand et al. (1983b) data also showed less of a response in females compared to males in terms TCE induction of liver weight at the 37 to 150 ppm range of exposure in NMRI strain. While the data for TCE dose-response of liver tumor induction is very limited, it is suggestive of a correlation of TCE-induced increases in liver weight correlating liver tumor induction with a pattern that is dissimilar to that of TCA.

Of those experiments conducted at ~1,000 mg/kg/d gavage dose of TCE in male B6C3F1 mice for at least 79 weeks (Bull et al., 2002; NCI, 1976; Anna et al., 1994; NTP, 1990) the control values were conducted in varying numbers of animals (some as low as $n = 15$, i.e., Bull et al., 2002) and with varying results. The incidence of hepatocellular carcinomas ranged from 1.2 to 16.7% (NCI, 1976; Anna et al., 1994, NTP, 1990) and the incidence of adenomas ranged from 1.2 to 14.6% (Anna et al., 1994; NTP, 1990) in control B6C3F1 mice. After ~1,000 mg/kg/d TCE treatment, the incidence of carcinomas ranged from 19.4 to 62% (Bull et al., 2002; NCI, 1976; Anna et al., 1994; NTP, 1990) with 3 of the studies (NCI, 1976; Anna et al., 1994; NTP, 1990) reporting a range of incidences between 42.8 to 62.0%). The incidence of adenomas ranged from 28 to 66.7% (Bull et al., 2002; Anna et al., 1994; NTP, 1990). These data are illustrative of the variability between experiments to determine the magnitude and nature of the TCE response in the same gender (male), strain (B6C3F1), time of exposure (3/4 studies were for 76−79 weeks and 1 for 2 years duration), and roughly the same dose (800−1,163 mg/kg/d TCE). Given, that the TCE-induced liver response, as measured by liver weight increase, is highly correlated with total oxidative metabolism to a number of agents that are hepatoactive agents and hepatocarcinogens, the variability in response from TCE exposure would be expected to be greater than studies of exposure to a single metabolite such as TCA or DCA.

Caldwell et al. (2008b) have commented on the limitations of experimental paradigms used to study liver tumor induction by TCE metabolites and show that 51-week exposure duration has consistently produced a tumor response for these chemicals, but with greater lesion incidence and multiplicity at 82 weeks. As reported by DeAngelo et al. (1999) and Pereira (1996), full expression of tumor induction in the mouse does not occur until 78 to 100 weeks of DCA or TCA exposure, especially at lower concentrations. Thus, use of abbreviated exposure durations and concurrently high exposure concentrations limits the ability of such experiments to detect a treatment-related effect with the occurrence of additional toxicity not necessarily associated with tumor-induction. Caldwell et al. (2008b) present a table that shows that the differences in the ability of the studies to detect treatment-related effects could also be attributed to a varying and low number of animals in some exposure groups and that because of the low numbers of animals tested at higher exposures, the power to detect a statistically significant change is very low and in fact for many of the endpoints is considerably less than "50% chance." Table E-17 from Caldwell et al. (2008b) illustrates the importance of experimental design and the limitations in many of the studies in the TCE metabolite database.

Table E-17. Power calculations[a] for experimental design described in text, using Pereira et al. as an example

Exposure concentration[b] in female B6C3F1 mice (Pereira, 1996;Pereira and Phelps, 1996)	Number of animals	Power calculation for foci	Power calculation for adenomas	Power calculation for carcinomas
20.0 mmol/L NaCl (control) (82 wks)	90	Null hypothesis	Null hypothesis	Null hypothesis
2.58 g/L DCA (82 wks)	19	0.03	0.03	0.13
0.86 g/L DCA (82 wks)	28	0.74	0.20	0.91
0.26 g/L DCA (82 wks)	50	0.99	0.98	–
3.27 g/L TCA (82 wks)	18	0.15	0.09	0.14
1.10 g/L TCA (82 wks)	27	0.60	0.64	0.3
0.33 g/L TCA (82 wks)	53	0.93	0.91	–

[a]The power calculations represent the probability of rejecting the null hypothesis when in fact the alternate hypothesis is true for tumor multiplicity (i.e., the total number of lesions/number of animals). The higher the power number calculated, the more confidence we have in the null hypothesis. Assumptions made included: normal distribution for the fraction of tumors reported, null hypothesis represents what we expected the control tumor fraction to be, the probability of a Type I error was set to 0.05, and the alternate hypothesis was set to four times the null hypothesis value.

[b]Conversion of mmol/L to g/L from the original reports of Pereira (1996) and Pereira and Phelps (1996) is as follows: 20.0 mmol/L DCA = 2.58 g/L, 6.67 mmol/L DCA = 0.86 g/L, 2.0 mmol/L = 0.26 g/L, 20.0 mmol/L TCA = 3.27 g/L, 6.67 mmol/L TCA = 1.10 g/L, 2.0 mmol/L TCA = 0.33 g/L.

Bull et al. (1990) examined male and female B6C3F1 mice (age 37 days) exposed from 15 to 52 weeks to neutralized DCA and TCA (1 or 2 g/L) but tumor data were not suitable for dose response. They reported effects of DCA and TCA exposure on liver weight and percent liver/body changes that gave a pattern of hepatomegaly generally consistent with short-term exposure studies. Only 10 female mice were examined at 52 weeks but the female mice were reported to be as responsive as males at the exposure concentration tested. After 37 weeks of treatment and then a cessation of exposure for 15 weeks, liver weights percent liver/body weight were reported to be elevated over controls which Bull et al. (1990) partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. Macroscopically, livers treated with DCA were reported to have multifocal areas of necrosis and frequent infiltration of lymphocytes on the surface and an interior of the liver. For TCA-treated mice, similar necrotic lesions were reported but at such a low frequency that they were similar to controls. Marked cytomegaly was reported from exposure to either 1 or 2 g/L DCA throughout the liver. Cell size was reported to be increased from TCA and DCA treatment with DCA producing the greatest change. The 2 g/L TCA exposures were observed to have increased accumulations of lipofuscin but no quantitative analysis was done. Photographs of light microscopic sections, that were supposed to be representative of DCA and TCA treated livers at 2 g/L, showed such great hepatocellular hypertrophy from DCA treatment that sinusoids were obscured. Such a degree of cytomegaly could have resulted in reduction of blood flow and contributed to focal necrosis observed at this level of exposure.

As discussed in Sections E.3.2 and E.3.4.2.1, glycogen accumulation has been described to be present in foci in both humans and animals as a result from exposure to a wide variety of carcinogenic agents and predisposing conditions in animals and humans. Bull et al (1990) reported that glycogen deposition was uniformly increased from 2 g/L DCA exposure with photographs of TCA exposure showing slightly less glycogen staining than controls. However, the abstract and statements in the paper suggest that there was increased PAS positive material from TCA treatment that has caused confusion in the literature in this regard. Kato-Weinstein et al. (2001) reported that in male B6C3F1 mice exposed to DCA and TCA, the DCA treatment increased glycogen and TCA decreased glycogen content of the liver by using both chemical measurement of glycogen in liver homogenates and by using ethanol-fixed sections stained with PAS, a procedure designed to minimize glycogen loss. Kato-Weinstein et al. (2001) reported that glycogen rich and poor cells were scattered without zonal distribution in male B6C3F1 mice exposed to 2 g/L DCA for 8 weeks. For TCA treatments they reported centrilobular decreases in glycogen and ~25% decreases in whole liver by 3 g/L TCA. Kato-Weinstein et al. (2001) reported whole liver glycogen to be increased ~1.50-fold of control (90 vs. 60 mg glycogen/g liver) by 2 g/L DCA after 8 weeks exposure male B6C3F1 mice with a maximal level of glycogen accumulation occurring after 4 weeks of DCA exposure. Pereira et al. (2004) reported that after 8 weeks of exposure to 3.2 g/L DCA liver glycogen content was 2.20-fold of control levels (155.7 vs. 52.4 mg glycogen/g liver) in female B6C3F1 mice. Thus, the baseline level of glycogen content reported by (~60 mg/g) and the increase in glycogen after DCA exposure was consistent between Kato-Weinstein et al. (2001) and Pereira et al. (2004). However, the increase in liver weight reported by Kato-Weinstein et al. (2001) of 1.60-fold of control percent liver/body weight cannot be accounted for by the 1.50-fold of control glycogen content. Glycogen content only accounts for 5% of liver mass so that 50% increase in glycogen cannot account for the 60% increase liver mass induced by 2 g/L DCA exposure for 8 weeks reported by Kato-Weinstein (2001). Thus, DCA-induced increases in liver weight are occurring from other processes as well. Carter et al. (2003) and DeAngelo et al. (1999) reported increased glycogen after DCA treatment at much lower doses after longer periods of exposure (100 weeks). Carter reported increased glycogen at 0.5 g/L DCA and DeAngelo et al. (1999) at 0.03 g/L DCA in mice. However, there is no quantitation of that increase.

The issues involving identification of MOA through tumor phenotype analysis are discussed in detail below for the more general case of liver cancer as well as for specific hypothesized MOAs (see Sections E.3.1.4, E.3.1.8, E.3.2.1, and E.3.4.1.5). For TCE and its metabolites, c-Jun staining, H-rats mutation, tincture, heterogeneity in dysplacity have been used to describe and differentiate liver tumors in the mouse.

Bull et al. (2002) reported 1,000 mg/kg TCE administered via gavage daily for 79 weeks in male B6C3F1 mice to produce liver tumors and also reported deaths by gavage error (6 out of 40 animals). The limitations of the experiment are discussed in Caldwell et al. (2008b). Specifically, for the DCA and TCA exposed animals, the experiment was limited by low statistical power, a relatively short duration of exposure, and uncertainty in reports of lesion prevalence and multiplicity due to inappropriate lesions grouping (i.e., grouping of hyperplastic nodules, adenomas, and carcinomas together as “tumors”), and incomplete histopathokolology determinations (i.e., random selection of gross lesions for histopathology examination). For the TCE results, a high prevalence (23/36 B6C3F1 male mice) of adenomas and hepatocellular carcinoma (7/36) was reported. For determinations of immunoreactivity to c-Jun, as a marker of differences in “tumor” phenotype, Bull et al. (2002) included all lesions in most of their treatment groups, decreasing the uncertainty of his findings. However, for immunoreactivity results hyperplastic nodules, adenomas, and carcinomas were grouped and thus, changes in c-Jun expression between the differing types of lesions were not determined. Bull et al. (2002) reported lesion reactivity to c-Jun antibody to be dependent on the proportion of the DCA and TCA administered after 52 weeks of exposure. Given alone, DCA was reported to produce lesions in mouse liver for which approximately half displayed a diffuse immunoreactivity to a c-Jun antibody, half did not, and none exhibited a mixture of the two. After TCA exposure alone, no lesions were reported to be stained with this antibody. When given in various combinations, DCA and TCA coexposure induced a few lesions that were only c-Jun+, many that were only c-Jun-, and a number with a mixed phenotype whose frequency increased with the dose of DCA. For TCE exposure of 79 weeks, TCE-induced lesions were reported to also have a mixture of phenotypes (42% c-Jun+, 34% c-Jun-, and 24% mixed) and to be most consistent with those resulting from DCA and TCA coexposure but not either metabolite alone.

Stauber and Bull (1997) exposed male B6C3F1 mice (7 weeks old at the start of treatment) to 2.0 g/L neutralized DCA or TCA in drinking water for 38 or 50 weeks, respectively and then exposed (n = 12) to 0, 0.02, 0.1, 0.5, 1.0, 2.0 g/L DCA or TCA for an additional 2 weeks. Foci and tumors were combined in reported results as “lesions” and prevalence rates were not reported. The DCA-induced larger “lesions” were reported to be more “uniformly reactive to c-Jun and c-Fos” but many nuclei within the lesions displaying little reactivity to c-Jun. Stauber and Bull (1997) stated that while most DCA-induced “lesions” were homogeneously immunoreactive to c-Jun and C-Fos (28/41 lesions), the rest were stained heterogeneously. For TCA-induced lesions, the authors reported no difference in staining between “lesions” and normal hepatocytes in TCA-treated animals. These results are slightly different that those reported by Bull et al. (2002) for DCA, who report c-Jun positive and negative foci in DCA-induced liver tumors but no mixed lesions. Because “lesions” comprised of foci and tumors, different stages of progression reported in these results. The duration of exposures also differed between DCA and TCA treatment groups that can affect phenotype. The shorter duration of exposure can also prevent full expression of the tumor response.

Stauber et al. (1998) presented a comparison of *in vitro* results with “tumors” from Stauber and Bull (1997) and note that 97.5% of DCA-induced “tumors” were c-Jun + while none of the TCA-induced “tumors” were c-Jun +. However, the concentrations used to give tumors *in vivo* for comparison with *in vitro* results were not reported. This appears to differ from the heterogeneity of result for c-Jun staining reported by Bull et al. (2002) and Stauber and Bull (1997). There was no comparison of c-Jun phenotype for spontaneous tumors with the authors stating that because of such short time, no control tumors results were given. However, the results of Bull et al. (2002) and Stauber and Bull (1997), do show TCA-induced lesions to be uniformly c-Jun negative and thus, the phenotypic marker was able to show that TCE-induced tumors were more like those induced by DCA than TCA.

The premise that DCA induced c-Jun positive lesions and TCA-induced c-Jun negative lesions in mouse liver was used as the rationale to study induction of “transformed” hepatocytes by DCA and TCE treatment *in vitro*. Stauber et al. (1998) isolated primary hepatocytes from 5−8 week old male B6C3F1 mice ($n = 3$) and subsequently cultured them in the presence of DCA or TCA. In a separate experiment 0.5 g/L DCA was given to mice as pretreatment for 2 weeks prior to isolation. The authors assumed that the anchorage-independent growth of these hepatocytes was an indication of an “initiated cell.” DCA and TCA solutions were neutralized before use. After 10 days in culture with DCA or TCA (0, 0.2, 0.5 and 2.0 mM), concentrations of 0.5 mM or more DCA and TCA both induced an increase in the number of colonies that was statistically significant, increased with dose with DCA, and slightly greater for DCA. In a time course experiment the number of colonies from DCA treatment *in vitro* peaked by 10 days and did not change through days 15−25 at the highest dose and, at lower concentrations of DCA, increased time in culture induced similar peak levels of colony formation by days 20−25 as that reached by 10 days at the higher dose. Therefore, the number of colonies formed was independent of dose if the cells were treated long enough *in vitro*. However, not only did treatment with DCA or TCA induce anchorage independent growth but untreated hepatocytes also formed larger numbers of colonies with time, although at a lower rate than those treated with DCA. The level reached by untreated cells in tissue culture at 20 days was similar to the level induced by 10 days of exposure to 0.5 mM DCA. The time course of TCA exposure was not tested to see if it had a similar effect with time as did DCA. The colonies observed at 10 days were tested for c-Jun expression with the authors noting that "colonies promoted by DCA were primarily c-Jun positive in contrast to TCA promoted colonies that were predominantly c-Jun negative." Of the colonies that arose spontaneously from tissue culture conditions, 10/13 (76.9%) were reported to be c-Jun +, those treated with DCA 28/34 (82.3%) were c-Jun +, and those treated with TCA 5/22 (22.7%) were c-Jun +. Thus, these data show heterogeneity in cell in colonies but with more were c-Jun + colonies occurring by tissue culture conditions alone and in the presence of DCA, rather than in the presence of TCA. The authors reported that with time (24, 48, 72, and 96 hours) of culture conditioning the number of c-Jun+ colonies was increased in untreated controls. The authors reported that DCA treatment delayed the increase in c-Jun+ expression induced by tissue culture conditions alone in untreated controls while TCA treatment was reported to not affect the increasing c-Jun+ expression that increased with time in tissue culture. This results seems paradoxical given that DCA induced a higher number of colonies at 10 days of tissue culture than TCA and that most of the colonies were c-Jun positive. The number of colonies was greater for pretreatment with DCA, but the magnitude of difference over the control level was the same after DCA treatment *in vitro* without and without pretreatment. As to the relationship of c-Jun staining and peroxisome proliferators as a class, as pointed out by Caldwell and Keshava (2006), although Bull et al. (2004) have suggested that the negative expression of c-*jun* in TCA-induced tumors may be consistent with a characteristic phenotype shown in general by peroxisome proliferators as a class, there is no supporting evidence of this.

An approach to determine the potential MOAs of DCA and TCA through examination of the types of tumors each "induced" or "selected" was to examine H-ras activation (Ferreira-Gonzalez et al., 1995; Anna et al., 1994; Bull et al., 2002; Nelson et al., 1990). This approach has also been used to try to establish an H-ras activation pattern for "genotoxic" and "nongenotoxic" liver carcinogens compounds and to make inferences concerning peroxisome proliferator-induced liver tumors. However, as noted by Stanley et al. (1994), the genetic background of the mice used and the dose of carcinogen may affect the number of activated H-ras containing tumors that develop. In addition, the stage of progression of "lesions" (i.e., foci vs. adenomas vs. carcinomas) also has been linked the observance of H-ras mutations. Fox et al.

(1990) note that tumors induced by phenobarbital (0.05% drinking water (H_2O), 1 year), chloroform (200 mg/kg corn oil gavage, 2 times weekly for 1 year) or Ciprofibrate (0.0125% diet, 2 years) had a much lower frequency of H-ras gene activation than those that arose spontaneously (2-year bioassays of control animals) or induced with the "genotoxic" carcinogen benzidine-2 hydrochloric acid (HCl; 120 ppm, drinking H_2O, 1 year) in mice. In that study, the term "tumor" was not specifically defined but a correlation between the incidence of H-ras gene activation and development of either a hepatocellular adenoma or hepatocellular carcinoma was reported to be made with no statistically significant difference between the frequency of H-ras gene activation in the hepatocellular adenomas and carcinomas. Histopathological examination of the spontaneous tumors, tumors induced with benzidine-2HCL, Phenobarbital, and chloroform was not reported to reveal any significant changes in morphology or staining characteristics. Spontaneous tumors were reported to have 64% point mutation in codon 61 (n = 50 tumors examined) with a similar response for Benzidine of 59% (n = 22 tumors examined), whereas for Phenobarbital the mutation rate was 7% (n = 15 tumors examined), chloroform 21% (n = 24 tumors examined) and Ciprofibrate 21% (n = 39 tumors examined). The Ciprofibrate-induced tumors were reported to be more eosinophilic as were the surrounding normal hepatocytes. Hegi et al. (1993) tested Ciprofibrate-induced tumors in the NIH3T3 cotransfection-nude mouse tumorigenicity assay, which the authors state is capable of detecting a variety of activated proto-oncogenes. The tumors examined (Ciprofibrate-induced or spontaneously arising) were taken from the Fox et al. study (1990), screened previously, and found to be negative for H-ras activation. With the limited number of samples examined, Hegi et al. concluded that ras proto-oncogene activation or activation of other proto-oncogenes using the nude mouse assay were not frequent events in Ciprofibrate-induced tumors and that spontaneous tumors were not promoted with it. Using the more sensitive methods, the H-ras activation rate was reported to be raised from 21 to 31% for Ciprofibrate-induced tumors and from 64 to 66% for spontaneous tumors. Stanley et al. (1994) studied the effect of methylclofenapate (MCP) (25 mg/kg for up to 2 years), a peroxisome proliferator, in B6C3F1 (relatively sensitive) and C57BL/10J (relatively resistant) mice for H-ras codon 61 point mutations in MCP-induced liver tumors (hepatocellular adenomas and carcinomas). In the B6C3F1 mice the number of tumors with codon 61 mutations was 11/46 and for C57BL/10J mice 4/31. Unlike the findings of Fox et al. (1990), Stanley et al. (1994) reported an increase in the frequency of mutation in carcinomas, which was reported to be twice that of adenomas in both strains of mice, indicating that stage of progression was related to the number of mutations in those tumors, although most tumors induced by MCP did not have this mutation.

In terms of liver tumor phenotype, Anna et al. (1994) reported that the H-ras codon 61 mutation frequency was not statistically different in liver tumors from DCA and TCE-treated mice from a highly variable number of tumors examined. In regard to mutation spectra in H-ras oncogenes in control or spontaneous tumors, the patterns were slightly different but mostly similar to that of DCA-induced tumors (0.5% in drinking water). From their concurrent controls they reported that H-ras codon 61 mutations in 17% (n = 6) of adenomas and 100% (n = 5) of carcinomas. For historical controls (published and unpublished) they reported mutations in 73% (n = 33) of adenomas and mutations in 70% (n = 30) of carcinomas. For tumors from TCE treated animals they reported mutations in 35% (n = 40) of adenomas and 69% (n = 36) of carcinomas, while for DCA treated animals they reported mutations in 54% (n = 24) of adenomas and in 68% (n = 40) of carcinomas. Anna et al. (1994) reported more mutations in TCE-induced carcinomas than adenomas.

The study of Ferreira-Gonzalez et al. (1995) in male B6C3 F1 mice has the advantage of comparison of tumor phenotype at the same stage of progression (hepatocellular carcinoma), for allowance of the full expression of a tumor response (i.e., 104 weeks), and an adequate number of spontaneous control lesions for comparison with DCA or TCA treatments. However, tumor phenotype at an endstage of tumor progression reflects of tumor progression and not earlier stages of the disease process. In spontaneous liver carcinomas, 58% were reported to show mutations in H-61 as compared with 50% of tumor from 3.5 g/L DCA-treated mice and 45% of tumors from 4.5.g/L TCA-treated mice. Thus, there was a heterogeneous response for this phenotypic marker for the spontaneous, DCA-, and TCA-treatment induced hepatocellular carcinomas and not a pattern of reduced H-ras mutation reported for a number of peroxisome proliferators. A number of peroxisome proliferators have been reported to have a much smaller mutation frequency that spontaneous tumors (e.g., 13−24% H-ras codon 61 mutations after Methylclofenopate depending on mouser strain, Stanley et al. [1994]: 21 to 31% for Ciprofibrate-induced tumors and from 64 to 66% for spontaneous tumors, Fox et al. [1990] and Hegi et al. [1993]).

Bull (2000) suggested that "the report by Anna et al (1994) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-ras oncogene than those observed in spontaneous tumors of control mice." Bull (2000) stated that "results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism" but went on to suggest that it is not possible to *a priori* rule out a role for selection in this process and that differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver tumors. Bull (2000) noted that data from Anna et al. (1994), Ferreira-Gonzalez et al. (1995), and Maronpot et al. (1995)

indicated that mutation frequency in DCA-induced tumors did not differ significantly from that observed in spontaneous tumors. Bull (2000) also noted that the mutation spectra found in DCA-induced tumors has a striking similarity to that observed in TCE-induced tumors, and DCA-induced tumors were significantly different than that of TCA-induced liver tumors.

Bull et al. (2002) reported that mutation frequency spectra for the H-ras codon 61 in mouse liver "tumors" induced by TCE (*n* = 37 tumors examined) were reported to be significantly different than that for TCA (*n* = 41 tumors examined), with DCA-treated mice tumors giving an intermediate result (*n* = 64 tumors examined). In this experiment, TCA-induced "tumors" were reported to have more mutations in codon 61 (44%) than those from TCE (21%) and DCA (33%). This frequency of mutation in the H-ras codon 61 for TCA is the opposite pattern as that observed for a number of peroxisome proliferators in which the number of mutations at H-ras 61 in tumors has been reported to be much lower than spontaneously arising tumors (see Section E.3.4.1.5). Bull et al. (2002) noted that the mutation frequency for all TCE,TCA or DCA tumors was lower in this experiment than for spontaneous tumors reported in other studies (they had too few spontaneous tumors to analyze in this study), but that this study utilized lower doses and was of shorter duration than that of Ferreira-Gonzalez et al. (1995). These are additional concerns in addition to the effects of lesion grouping in which a lower stage of progression is group with more advanced stages. In a limited subset of tumors that were both sequenced and characterized histologically, only 8 of 34 (24%) TCE-induced adenomas but 9/15 (60%) of TCE-induced carcinomas were reported to have mutated H-ras at codon 61, which the authors suggest is evidence that this mutation is a late event.

Thus, in terms of H-ras mutation, the phenotype of TCE-induced tumors appears to be more like DCA-induced tumors (which are consistent with spontaneous tumors), or those resulting from a coexposure to both DCA and TCA (Bull et al., 2002), than from those induced by TCA. As noted above, Bull et al. (2002) reported the mutation frequency spectra for the H-ras codon 61 in mouse liver tumors induced by TCE to be significantly different than that for TCA, with DCA-treated mice tumors giving an intermediate result and for TCA-induced tumors to have a H-ras profile that is the opposite than those of a number of other peroxisome proliferators. More importantly, these data suggest that using measures, other than dysplasticity and tincture, mouse liver tumors induced by TCE are heterogeneous in phenotype.

With regard to tincture, Stauber and Bull (1997) reported the for male B6C3F1 mice, DCA-induced "lesions" contained a number of smaller lesions that were heterogeneous and more eosinophilic with larger "lesions" tending to less numerous and more basophilic. For TCA results using this paradigm, the "lesions" were reported to be less numerous, more basophilic, and larger than those induced by DCA. Carter et al. (2003) used tissues from the DeAngelo et al.

(1999) and examined the heterogeneity of the DCA-induced lesions and the type and phenotype of preneoplastic and neoplastic lesions pooled across all time points. Carter et al. (2003) examined the phenotype of liver tumors induced by DCA in male B6C3 F1 mice and the shape of the dose-response curve for insight into its MOA. They reported a dose-response of histopathologic changes (all classes of premalignant lesions and carcinomas) occurring in the livers of mice from 0.05−3.5 g/L DCA for 26−100 weeks and suggest foci and adenomas demonstrated neoplastic progression with time at lower doses than observed DCA genotoxicity. Preneoplastic lesions were identified as eosinophilic, basophilic and/or clear cell (grouped with clear cell and mixed cell) and dysplastic. Altered foci were 50% eosinophilic with about 30% basophilic. As foci became larger and evolved into carcinomas they became increasingly basophilic. The pattern held true through out the exposure range. There was also a dose and length of exposure related increase in atypical nuclei in "noninvolved" liver. Glycogen deposition was also reported to be dose-dependent with periportal accumulation at the 0.5 g/L exposure level. Carter et al. (2003) suggested that size and evolution into a more malignant state are associated with increasing basophilia, a conclusion consistent with those of Bannasch (1996) and that there a greater periportal location of lesions suggestive as the location from which they arose. Consistent with the results of DeAngelo et al. (1999), Carter et al. (2003) reported that DCA (0.05−3.5 g/L) increased the number of lesions per animal relative to animals receiving distilled water, shortened the time to development of all classes of hepatic lesions, and that the phenotype of the lesions were similar to those spontaneously arising in controls. Along with basophilic and eosinophilic lesions or foci, Carter et al. (2003) concluded that DCA-induced tumors also arose from isolated, highly dysplastic hepatocytes in male B6C3F1 mice chronically exposed to DCA suggesting another direct neoplastic conversion pathway other than through eosinophilic or basophilic foci.

Rather than male B6C3F1 mice, Pereira (1996) studied the dose-response relationship for the carcinogenic activity of DCA and TCA and characterized their lesions (foci, adenomas and carcinomas) by tincture in females (the generally less sensitive gender). Like the studies of TCE by Maltoni et al. (1986), female mice were also reported to have increased liver tumors after TCA and DCA exposures. Pereira (1996) pool lesions were pooled for phenotype analysis so the affect of duration of exposure could not be determined nor adenomas separated from carcinomas for "tumors." However, as the concentration of DCA was decreased the number of foci was reported by Pereira (1996) to be decreased but the phenotype of the foci to go from primarily eosinophilic foci (i.e., ~95% eosinophilic at 2.58 g/L DCA) to basophilic foci (~57% eosinophilic at 0.26 g/L). For TCA the number of foci was reported to ~40 basophilic and ~60 eosinophilic regardless of dose. Spontaneously occurring foci were more basophilic by a ratio of 7/3. Pereira (1996) described the foci of altered hepatocytes and tumors induced by DCA in female B6C3F1 mice to be eosinophilic at higher exposure levels but at lower or intermittent exposures to be half eosinophilic and half basophilic. Regardless of exposure level, half of the TCA-induced foci were reported to be half eosinophilic and half basophilic with tumors 75% basophilic. In control female mice, the limited numbers of lesions were mostly basophilic, with most of the rest being eosinophilic with the exception of a few mixed tumors. The limitations of descriptions tincture and especially for inferences regarding peroxisome proliferator from the description of "basophilia" is discussed in Section E.3.4.1.5.

The results appear to differ between male and female B6C3F1 mice in regard to tincture for DCA and TCA at differing doses. What is apparent is that the tincture of the lesions is dependent on the stage of tumor progression, agent (DCA or TCA), gender, and dose. Also what is apparent from these studies is the both DCA and TCA are heterogeneous in their tinctoral characteristics as well as phenotypic markers such as mutation spectra or expression of c-Jun.

The descriptions of tumors in mice reported by the NCI, NTP, and Maltoni et al. studies are also consistent with phenotypic heterogeneity as well as consistency with spontaneous tumor morphology (see Section E.3.4.1.5). As noted in Section E.3.1, hepatocellular carcinomas observed in humans are also heterogeneous. For mice, Maltoni et al. (1986) described malignant tumors of hepatic cells to be of different subhistotypes, and of various degrees of malignancy and were reported to be unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype tumors were described as usual type observed in Swiss and B6C3F1 mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. For the NCI (1976) study, the mouse liver tumors were described in detail and to be heterogeneous "as described in the literature" and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure. The NTP (1990) study reported TCE exposure to be associated with increased incidence of hepatocellular carcinoma (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. Hepatocellular carcinomas were reported to have markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies and variations in nuclear appearance. Furthermore, in many instance several or all of the abnormalities were reported to be present in different areas of the tumor and variations in architecture with some of the hepatocellular carcinomas having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors from all 3 of these studies.

Caldwell and Keshava (2006) report

> that Bannasch (2001) and Bannasch et al. (2001) describe the early phenotypes of preneoplastic foci induced by many oncogenic agents (DNA-reactive chemicals, radiation, viruses, transgenic oncogenes and local hyperinsulinism) as insulinomimetic. These foci and tumors have been described by tincture as eosinophilic and basophilic and to be heterogeneous. The tumors derived from them after TCE exposure are consistent with the description for the main tumor lines of development described by Bannasch et al (2001) (see Section 3.4.1.5). Thus, the response of liver to DCA (glycogenosis with emergence of glycogen poor tumors) is similar to the progression of preneoplastic foci to tumors induced from a variety of agents and conditions associated with increased cancer risk.

Furthermore Caldwell and Keshava (2006) note that Bull et al. (2002) report expression of insulin receptor (IR) to be elevated in tumors of control mice or mice treated with TCE, TCA and DCA but not in nontumor areas suggesting that this effect is not specific to DCA.

There is a body of literature that has focused on the effects of TCE and its metabolites after rats or mice have been exposed to “mutagenic” agents to “initiate” hepatocarcinogenesis and this is discussed in Section E.4.2, below. TCE and its metabolites were reported to affect tumor incidence, multiplicity, and phenotype when given to mice as a coexposure with a variety of “initiating” agents and with other carcinogens. Pereira and Phelps (1996) reported that MNU alone induced basophilic foci and adenomas. MNU and low concentrations of DCA or TCA in female mice were reported to induce heterogeneous for foci and tumor with a higher concentration of DCA inducing more eosinophilic and a higher concentration of TCA inducing more tumors that were basophilic. Pereira et al. (2001) reported that not only dose, but gender also affected phenotype in mice that had already been exposed to MNU and were then exposed to DCA. As for other phenotypic markers, Lantendresse and Pereira (1997) reported that exposure to MNU and TCA or DCA induced tumors that had some commonalities, were heterogeneous, but for female mice were overall different between DCA and TCA as coexposures with MNU.

Stop experiments which attempt to ascertain the whether progression differences exist between TCA and DCA have used higher concentrations at much lower durations of exposure. A question arises as to whether the differences in results between those animals in which treatment was suspended in comparison to those in which had not had been conducted so that full expression of response had not been allowed rather than "progression" as well as the effects of using large doses. After 37 weeks of treatment and then a cessation of exposure for 15 weeks Bull et al. (1990) reported that after 15 weeks of cessation of exposure, liver weight and percent liver/body weight were reported to still be statistically significantly elevated after DCA or TCA treatment. The authors partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. In terms of liver tumor induction, the authors stated that "statistical analysis of tumor incidence employed a general linear model ANOVA with contrasts for linearity and deviations from linearity to determine if results from groups in which treatments were discontinued after 37 weeks were lower than would have been predicted by the total dose consumed." The multiplicity of tumors observed in male mice exposed to DCA or TCA at 37 weeks and then sacrificed at 52 weeks were reported by the authors to have a response in animals that received DCA very close to that which would be predicted from the total dose consumed by these animals. The response to TCA was reported by the authors to deviate significantly ($p = 0.022$) from the linear model predicted by the total dose consumed. Multiplicity of lesions per mouse and not incidence was used as the measure. Most importantly the data used to predict the dose response for "lesions" used a different methodology at 52 weeks than those at 37 weeks. Not only were not all animal's lesions examined, but foci, adenomas, and carcinomas were combined into one measure. Therefore, foci, of which a certain percentage have been commonly shown to spontaneously regress with time, were included in the calculation of total "lesions." Pereira and Phelps (1996) note that in MNU-treated mice that were then treated with DCA, the yield of altered hepatocytes decreases as the tumor yields increase between 31 and 51 weeks of exposure suggesting progression of foci to adenomas. Initiated and noninitiated control mice were reported to also have fewer foci/mouse with time. Because of differences in methodology and the lack of discernment between foci, adenomas, and carcinomas for many of the mice exposed for 52 weeks, it is difficult to compare differences in composition of the "lesions" after cessation of exposure in the Bull et al. (1990) study. For TCA treatment the number of animals examined for determination of which "lesions" were foci, adenomas, and carcinomas was 11 out of the 19 mice with "lesions" at 52 weeks while all 4 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. For DCA treatment the number of animals examined was only 10 out of 23 mice with "lesions" at 52 weeks while all 7 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. Most importantly, when lesions were examined microscopically then did not all turn out to be preneoplastic or neoplastic. Two lesions appeared "to be histologically normal" and one necrotic. Not only were a smaller number of animals examined for the cessation exposure than continuous exposure but only the 2 g/L exposure levels of DCA and TCA were studied for cessation. The number of animals bearing "lesions" at 37 and then 15 week cessation weeks was 7/11 (64%) while the number of animals bearing lesions at 52 weeks was 23/24 (96%) after 2 g/L DCA exposure. For TCA the number of animals bearing lesions at 37 weeks and then 15 weeks cessation was 4/11 (35%) while the number of animals bearing lesions at 52 weeks was 19/24 (80%). While suggesting that cessation of exposure diminished the number of "lesions," conclusions regarding the identity and progression of those lesion with continuous versus noncontinuous DCA and TCA treatment are tenuous.

E.2.5. Studies of Chloral Hydrate (CH)

Given that total oxidative metabolism appears to be highly correlated with TCE-induced increases in liver weight in the mouse rather than merely the presence of TCA, other metabolites are of interest as potential agents mediating the effects observed for TCE. Recently Caldwell and Keshava provided a synopsis of the results of more recent studies involving CH (Caldwell and Keshava, 2006). A large fraction of TCE oxidative metabolism appears to go through CH, with subsequent metabolism to TCA and trichloroethanol (Chiu et al., 2006b). Merdink et al. (2008) demonstrated that CH administered to humans can be extremely variable and complex in its pharmacokinetic behavior with a peak plasma concentration of CH in plasma 40−50 times higher than observed at the same time interval for other subjects. Studies of CH toxicity in rodents are consistent with the general presumption that oxidative metabolites are important for TCE-induced liver tumors, but whether CH and its metabolites are sufficient to explain all of TCE liver tumorigenesis remains unclear, particularly because of uncertainties regarding how DCA may be formed (Chiu et al., 2006b). Studies of CH may enable a comparison between toxicity of TCE and CH and may help elucidate its role in TCE effects. As with other TCE metabolites, the majority of the studies have focused on the mouse liver tumor response. For rats, while the limited data suggests that there is less of a response than mice to CH, those studies are limited in power or reporting.

Daniel et al. (1992) exposed adult male B6C3F1 (C57B1/6jC male mice bred to C3Heb/Fej female mice) 28-day old mice to CH, 2-chloroacetaldehyde, or DCA in 2 different phases (I and II) with initial weights ranging from 9.4 to 13.6 g. The test compounds were buffered and administered in drinking water for 30 and 60 weeks (n = 5 for interim sacrifice), and for 104 weeks (n = 40). The concentration of CH was 1 g/L and for DCA 0.5 g/L and the estimated doses of DCA were 85, 93, and 166 mg/kg/d for the DCA group I, DCA group II, and CH exposed group, respectively. Microscopic examination of tissues was conducted for all tissues for five animals of the CH groups with liver, kidneys, testes, and spleen, in addition to all gross lesions, reported to be examined microscopically in all of the 104-week survivors. The initial body weight for drinking water controls was reported to be 12.99 ± 3.04 g for group I (n = 23) and 10.48 ± 1.70 for group II (n = 10). For DCA treated animals, initial body weights were 13.44 ± 2.57 g for group I (n = 23) and 9.65 ± 2.72 g for group II (n = 10). For the CH treated group the initial body weights were reported to be 10.42 ± 2.49 g (n = 40). It is not clear from the report what control group best matched, if any, the CH group. Thus, the mean initial body weights of the groups as well as the number of animals varied considerably in each group (i.e., ~40% difference in mean body weights at the beginning of the study). The number of animals surviving till the termination of the experiment was 10, 10, 16, 8, and 24 for the control group I, control group II, DCA group I, DCA group II, and CH groups, respectively. An increase in absolute and relative liver weight versus reported to be observed at 30 weeks for DCA and CH groups and at 60 weeks for CH but data were not shown in the study. At 104 weeks, the data for the surviving control groups were combined as was that for the 2 DCA treatment groups. Of note was that for CH treated survivors (n = 24) water consumption was significantly reduced in comparison to controls. Absolute liver weight was reported to be 2.09 ± 0.6 g, 3.17 ± 1.3 g and 2.87 ± 1.1 g for control, DCA and CH treatment groups, respectively. The % liver to body weight was reported to be similarly elevated (1.57-fold of control for DCA and 1.41-fold of control for CH) at 104 weeks. At 104 weeks the treatment-related liver lesions in histological sections were reported to be most prominently hepatocytomegaly and vacuolization in DCA-treated animals. Cytomegaly was also reported to be in 5, 92, and 79% of control, DCA and CH treatment groups, respectively. Cytomegaly in CH treated mice was described as minimal and associated with an increased number of basophilic granules (rough endoplasmic reticulum). Hepatocellular necrosis and chronic active inflammation were reported to be mildly increased in both prevalence and severity in all treated groups. The histological findings, from interim sacrifices (n = 5), were considered by the authors to be unremarkable and were not reported. Liver tumors were increased by DCA and CH treatment. The percent incidence of liver carcinomas and adenomas combined in the surviving animals was 15, 75, and 71% in control, DCA and CH treated mice, respectively. In the CH treated group, the incidence of hepatocellular carcinoma was 46%. The number of tumors/animals was also significantly increased with CH treatment. Most importantly, morphologically the authors noted that there did not appear to be any discernable differences in the visual appearance of the DCA- and CH-induced tumors.

George et al. (2000) exposed male B6C3F1 mice and male F344/N rats to CH in drinking water for 2 years (up to 162.6 mg/kg/d). Target drinking water concentrations were 0, 0.05, 0.5, and 2 g/L CH in rats and 0, 0.05, 0.5 and 1.0 g/L CH in mice. Groups of animals (*n* = 6/group) were sacrificed at 13 (rats only), 26, 52 and 78 weeks following the initiation of dosing with terminal sacrifices at Week 104. A complete pathological examination was performed on 5 rats and mice from the high-dose group, with examination primarily of gross lesions except for liver, kidney, spleen and testes. BrdU incorporation was measured in the interim sacrifice groups in rats and mice with PCO examined at 26 weeks in mice. In rats, the number of animals surviving >78 weeks and examined for hepatocellular proliferative lesions was 42, 44, 44, and 42 for the control, 7.4, 37.4 and 163.6 mg/kg/d CH treatment groups, respectively. Only 32, 36, 35, and 32 animals were examined at the final sacrifice time. Only the lowest treatment group had increased liver tumors, which were marginally significantly increased by treatment. The percent of animals with hepatocellular adenomas and carcinomas was reported to be 2.4, 14.3, 2.3 and 6.8% in male rats. In mice, preneoplastic foci and adenomas were reported to be increased in the livers of all CH treatment groups (13.5−146.6 mg/kg/d) at 104 weeks. The incidences of adenomas were reported to be statistically increased at all dose levels, the incidences of carcinomas significantly increased at the highest dose, and time-to-tumor decreased in all CH-treatment groups. The percent incidence of hepatocellular adenomas was reported to be 21.4, 43.5, 51.3, and 50% in control, 13.5, 65.0, and 146.6 mg/kg day treatment groups, respectively. The percent incidence of hepatocellular carcinomas was reported to be 54.8, 54.3, 59.0, and 84.4% in these same groups. The resulting percent incidence of hepatocellular adenomas and carcinomas was reported to be 64.3, 78.3, 79.5, and 90.6%. The number of mice surviving >78 weeks was reported to be 42, 46, 39, and 32 and the number surviving to final sacrifice to be 34, 42, 31, and 25 for control, 13.5, 65.0 and 146.56 mg/kg/d, respectively. CH exposure was reported to not alter serum chemistry, hepatocyte proliferation (i.e., DNA synthesis), or hepatic PCO activity (an enzyme associated with PPARα agonism) in rats and mice at any of the time periods monitored (all interim sacrifice periods for BrdU incorporation, 52 or 78 weeks for serum enzymes, and 26 weeks for PCO) with the exception of 0.58 g/L CH at 26 weeks slightly increasing hepatocyte labeling (~2−3-fold increase over controls) in rats and mice but the percent labeling still represented 3% or less of hepatocytes. With regard to other carcinogenic endpoints only five animals were examined at the high dose, thereby limiting the study's power to determine an effect. Control mice were reported to have a high spontaneous carcinoma rate (54%), thereby limiting the ability to detect a treatment-related response. No descriptions of the foci or tumor phenotype were given. However, of note is the lack of induction of PCO response with CH at 26 weeks of administration in either rats or mice.

Leakey et al. (2003a) studied the effects of CH exposure (0, 25, 50, and 100 mg/kg, 5 days/week, 104−105 weeks via gavage) in male B6C3F1 mice with dietary control used to manipulate body growth (n = 48 for 2 year study and n = 12 for the 15-month interim study). Dietary control was reported to decrease background liver tumor rates (incidence of 15−20%) and was reported to be associated with decreased variation in liver-to-body weight ratios, thereby potentially increasing assay sensitivity. In dietary-controlled groups and groups fed *ad libitum*, liver adenomas and carcinomas (combined) were reported to be increased with CH treatment. With dietary restriction there was a more discernable CH tumor-response with overall tumor incidence reduced, and time-to-tumor increased by dietary control in comparison to ad libitum fed mice. Incidences of hepatocellular adenoma and carcinoma overall rates were reported to be 33, 52, 49, and 46% for control, 25, 50, and 100 mg/kg ad libitum-fed mice, respectively. For dietary controlled mice the incidence rates were reported to be 22.9, 22.9, 29.2, and 37.5% for controls, 25, 50, and 100 mg/kg CH, respectively. Body weights were matched and carefully controlled in this study.

After 2 years of CH treatment the heart weights of ad libitum-fed male mice administered 100 mg/kg CH were reported to be significantly less and kidney weights of the 50 and 100 mg/kg less than vehicle controls. No other significant organ weight changes due to CH treatment were reported to be observed in either diet group except for liver. The liver weights of CH treated groups for by dietary groups were reported to be increased at 2 years and the absolute liver weights of dosed groups to be generally increased at 15 months with percent liver/body weight ratios increased in CH treated dietary-controlled mice at 15 months. There was 1.0-, 0.87-, and 1.08-fold of control percent liver/body weight for ad libitum fed mice exposed to 25, 50, and 100 mg/kg CH, respectively. For dietary controlled mice, there was 1.05-, 1.08-, and 1.11-fold of control percent liver/body weight for the same dose groups at 15 months. Thus, there was no corresponding dose-response for percent liver/body weight in the ad libitum-fed mice, which were reported to show a much larger variation in liver-to-body-weight ratios (i.e., the standard deviation and standard errors were 2- to 17-fold lower in dietary controlled groups than for ad libitum-fed groups). Liver weight increases at 15-months did not correlate with 2-year tumor incidences with this group. However, for dietary controlled groups the increase in percent liver/body weights at 15 months were generally correlated with increases in liver tumors at 2 years. The incidences of peripheral or focal fatty change were reported to be increased in all CH-treated groups of ad libitum-fed mice at 15 months (approximately half the animals showed these changes for all dose groups, with no apparent dose-response). Of the enzymes associated with PPARα agonism (total CYP, CYP2B isoform, CYP4A, or lauric acid β-hydroxylase activity), only CYP4A and lauric acid β-hydroxylase activity were significantly increased at

15 months of exposure in the dietary-restricted group administered 100 mg/kg CH with no other groups reported showing a statistically significant increased response (n = 12/group). Although not statistically significant, the 100 mg/kg CH exposure group of ad libitum-fed mice also had an increase in CYP4A and lauric acid β-hydroxylase activity. The authors reported that the increase in magnitude of CYP4A and lauric acid β-hydroxylase activity at 100 mg/kg CH at 15 months in dietary controlled mice correlated with the increase incidence of mice with tumors. However, there was no correlation of tumor incidence and the increased enzyme activity associated with peroxisome proliferation in the ad libitum-fed mice. No descriptions of liver pathology were given other than incidence of mice with fatty liver changes. Hepatic malondialdehyde concentration in ad libitum fed and dietary controlled mice did not change with CH exposure at 15 months but the dietary controlled groups were all approximately half that of the ad libitum-fed mice. Thus, while overall increased tumors observed in the ad libitum diet correlated with increased malondialdehyde concentration, there was no association between CH dose and malondialdehyde induction for either diet.

Induction of peroxisome-associated enzyme activities was also reported for shorter times of CH exposure. Seng et al. (2003) described CH toxicokinetics in mice at doses up to 1,000 mg/kg/d for 2 weeks with dietary control and caloric restriction slightly reducing acute toxicity. Lauric acid β-hydroxylase and PCO activities were reported to be induced only at doses >100 mg/kg in all groups, with dietary-restricted mice showing the greatest induction. Differences in serum levels of TCA, the major metabolite remaining 24 hr after dosing, were reported not to correlate with hepatic lauric acid β-hydroxylase activities across groups.

Leuschner and Beuscher (1998) examined the carcinogenic effects of CH in male and female S-D rats (69−79 g, 25−29 days old at initiation of the experiment) administered 0, 15, 45, and 135 mg/kg CH in unbuffered drinking water 7 days/week (n = 50/group) for 124 weeks in males and 128 weeks in females. Two control groups were noted in the methods section without explanation as to why they were conducted as two groups. The mean survival for males was similar in treated and control groups with 20, 24, 20, 24, and 20% of Ccontrol I, Control II, 15, 45, and 135 mg/kg CH-treated groups, respectively, surviving till the end of the study. For female rats, the percent survival was 12, 30, 24, 28, and 16% for of Control I, Control II, 15, 45, and 135 mg/kg CH-treated groups, respectively. The authors report no substance-related influence on organ weights and no macroscopic evidence of tumors or lesions in male or female rats treated with CH for 124 or 128 weeks. However, no data are presented on the incidence of tumors using this paradigm, especially background rates. The authors report a statistically significant increase in the incidence of hepatocellular hypertrophy in male rats at the 135 mg/kg dose (14/50 animals vs. 4/50 and 7/50 in controls I and II). For female rats, the incidence of hepatocellular hypertrophy was reported to be 10/50 rats (Control I) and 16/50 (Control II) rats with 18/50, 13/50 and 12/50 female rats having hepatocellular hypertrophy after 15, 45, and 135 mg/kg CH, respectively. The lack or reporting in regard to final body weights, histology, and especially background and treatment group data for tumor incidences, limit the interpretation of this study. Whether this paradigm was sensitive for induction of liver cancer cannot be determined.

From the CH studies in mice, there is an apparent increase in liver adenomas and carcinomas induced by CH treatment by either drinking water or gavage with all available studies performed in male B6C3F1 mice. However, the background levels of hepatocellular adenomas and carcinomas in these mice in George et al. (2000) and body weight data from this study show it is from a tumor prone mouse. Comparisons with concurrent studies of mice exposed to DCA revealed that while both CH and DCA induced hepatomegaly and cytomegaly, DCA-induced cytomegaly was accompanied by vacuolization while that of CH to be associated with increased number of basophilic granules (rough endoplasmic reticulum) which would suggest separate effects. However, the morphology of the CH-induced tumors was reported to be similar between DCA and CH-induced tumors (Daniel et al., 1992). Using a similar paradigm (2-year study of B6C3F1 male mice), De Angelo et al. (1999) and Carter et al. (2003) described DCA-induced tumors to be heterogeneous. This is the same description given for TCE-induced tumors in the studies by NTP, NCI, and Maltoni et al. and to be a common description for tumors caused by a variety of carcinogenic agents. Similar to the studies cited above for CH, DeAngelo et al. (1999) reported that PCO levels were only elevated at 26 weeks at 3.5 g/L DCA and had returned to control levels by 52 weeks. Similar to CH, no increased tritiated thymidine was reported for DCA at 26 and 52 weeks with only 2-fold of control values reported at 0.05 g/L at 4 weeks. Leakey et al. (2003a) reported that ad libitum fed male mice exhibited a similar degree of increased incidence of peripheral or focal fatty change at 15 months for all CH doses but not enzymes associated with peroxisome proliferation. While dietary restriction seemed to have decreased background levels of tumors and increased time-to-tumor, CH-gave a clear dose-response in dietary restricted animals. However, while the overall level of tumor induction was reduced there was a greater induction of PPARα enzymes by CH. Induction of liver tumors by CH observed in ad libitum fed mice were not correlated with PPARα induction, with dietary restriction alone appearing to have greater levels of lauric acid ω-hydrolase activity in control mice at 15 months. Seng et al. (2003) report that lauric acid β-hydroxylase and PCO were induced only at exposure levels >100 mg/kg CH, again with dietary restricted groups showing the greatest induction. Such data argues against the role of peroxisome proliferation in CH-liver tumor induction in mice.

E.2.6. Serum Bile Acid Assays

Serum bile acids (SBA) have been suggested as a sensitive indicator of hepatotoxicity to a variety of halogenated solvents with an advantage of increased sensitivity and specificity over conventional liver enzyme tests that primarily reflect the acute perturbation of hepatocyte membrane integrity and "cell leakage" rather than liver functional capacity (i.e., uptake, metabolism, storage, and excretion functions of the liver) (Bai et al., 1992b; Neghab et al., 1997). While some studies have reported negative results, a number of studies have reported elevated SBA in organic solvent-exposed workers in the absence of any alterations in normal liver function tests. These variations in results have been suggested to arise from failure of some methods to detect some of the more significantly elevated SBA and the short-lived and reversible nature of the effect (Neghab et al., 1997). Neghab et al. (1997) have reported that occupational exposure to 1,1,2-trichloro-1,2,2-trifluoroethane and trichloroethylene has resulted in elevated SBA and that several studies have reported elevated SBA in experimental animals to chlorinated solvents such as carbon tetrachloride, chloroform, hexachlorobutadiene, tetrachloroethylene, 1,1,1-trichloroethane, and trichloroethylene at levels that do not induce hepatotoxicity (Bai et al., 1992a, b; Hamdan and Stacey, 1993; Wang and Stacey, 1990). Toluene, a nonhalogenated solvent, has also been reported to increase SBA in the absence of changes in other hepatobiliary functions (Neghab and Stacey, 1997). Thus, disturbance in SAB appears to be a generalized effect of exposure to chlorinated solvents and nonchlorinated solvents and not specific to TCE exposure.

Neghab et al. (1997) reported that 8 hour time-weighted averages exposure to TCE of 8.9 ppm, measured in the breathing zone using a charcoal tube personal sampler for the whole mean duration of exposure of 3.4 years, to have not significant changes in albumin, bilirubin, alkaline phosphatase, alanine aminotransferase, 5'-nucleosidase, γ-glutamyltransferase, but to have significantly increased total serum bile acids. Not only were total bile acids significantly increased in these TCE-exposed workers compared to controls (~2-fold of control), but, specifically, deoxycholic acid and subtotal of free bile acids were increased. Neghab et al. (1997) do not show the data, but also report that "despite the apparent overall low level of exposure, there was a very good correlations ($r = 0.94$) between the degree of increase in serum concentration of total bile acids and level of TCE." Neghab et al. (1997) note that while a sensitive indicator or exposure to such solvents in asymptomatic workers, there is no indication that actual liver injury occurs in conjunction with SAB increases.

Wang and Stacey (1990) administered TCE in corn oil via i.p. injection to male S-D rats (300−500 g) at concentrations of 0.01, 0.1, 1, 5, and 10 mmol/kg on 3 consecutive days (n = 4, 5, or 6) with liver enzymes and SBA examined 4 hours after the last TCE treatment. At these dose, there were not differences between treated and control animals in regard to alkaline phosphatase and sorbitol dehydrogenase concentrations and an elevation of alanine aminotransferase only at the highest dose. However, there was generally a reported dose-related increase in cholic acid, chenodeoxycholic acid, deoxycholic acid, taurocholic acid, tauroursodeoxycholic acid with cholic acid and taurochlolic acid increased at the lowest dose. The authors report that "examination of liver sections under light microscopy yielded no consistent effects that could be ascribed to trichloroethylene." In the same study a rats were also exposed to TCE via inhalation ($n = 4$) at 200 ppm for 28 days, and 1,000 ppm for 6 hours/day. Using this paradigm, cholic acid and taurocholic acid were significantly elevated at the 200 ppm level, (~10- and ~5-fold of control, respectively) with very large standard errors of the mean. At the 1,000 ppm level (6 hours, day) cholic acid and taurocholic acid were elevated to ~2-fold of control but neither was statistically significant. The large variability in responses between rats and the low number of rats tested in this paradigm limit its ability to determine quantitative differences between groups. Nevertheless, without the complications associated with i.p. exposure (see Section E.2.2.1, above), both inhalation exposure of TCE at a relative low exposure level was also associated with increased SBA levels. The authors stated that "no increases in alanine amino transferase levels were observed in the rats exposed to trichloroethylene via inhalation." No histopathology results were reported for rats exposed via inhalation. As stated by Wang and Stacey (1990), "intraperitoneal injection is not particularly relevant to humans" which was the rationale given for the inhalation exposure experiments in the study. They point out that intestinal interactions require consideration because a major determinant of SBA is their absorption from the gut and intestinal flora may play a role in bile acid metabolism. They also note that grooming done by the experimental rats would probably give small exposure via ingestion of TCE as well. However, Wang and Stacey (1990) reported consistent results in terms of TCE-induced changes in SBA at relatively low concentrations by either inhalation or i.p. routes of exposure that were not associated with other measures of toxicity.

Hamdan and Stacey (1993) administered TCE in corn oil (1 mmol/kg) in male Sprague Dawley rats (300−400 g) and followed the time-course of SBA elevation, TCE concentration and trichloroethanol in the blood at 2, 4, 8, and 16 hours after dosing ($n = 4,5$, or 6 per group). Liver and blood concentration of TCE were reported to peak at 4 hours while those of trichloroethanol peaked at 8 hours after dosing. TCE levels were not detectable by 16 hours in either blood or liver while those of trichloroethanol were still elevated. Elevations of SBA were reported to parallel those of TCE with cholic acid and taurochloate acid reported to show the highest levels of bile acids. The dose given was based on that reported by Wang and Stacey (1990) to give no hepatotoxicity but an increase in SBA. The authors state that liver injury parameters were checked and found unaffected by TCE exposure but do not show the data. Thus, it was TCE concentration and not that of its metabolite that was most closely related to changes in SBA and after a single exposure, the effect was reversible. In an *in vitro* study by Bai and Stacey (1993), TCE was studied in isolated rat hepatocytes with TCE reported to cause a dose-related suppression of initial rates of cholic acid and taurocholic acid but with no significant effects on enzyme leakage and intracellular calcium contents, further supporting a role for the parent compound in this effect. The authors noted that the changes in SBA result from interference with a physiological process rather "than an event associated with significant pathological consequences."

E.3. STATE OF SCIENCE OF LIVER CANCER MODES OF ACTION (MOAs)

The experimental evidence in mice shows that TCE and its metabolites induce foci, hepatocellular adenomas, and carcinomas that are heterogeneous in nature as indicated by phenotypic differences in tincture, mutational markers, or gene expression markers. The tumors induced by TCE are reflective of phenotypes that are either similar to those induced by mixtures of DCA and TCA exposure, or more like those induced by DCA. These tumors have been described to be similar also to those arising spontaneously in mice or from chemically induced hepatocarcinogenesis and to arise from preneoplastic foci, and in the case of DCA, single dysplastic hepatocytes as well as foci. HCC observed in humans also has been described to be heterogeneous and to be associated with formation of preneoplastic nodules. Although several conditions have been associated with increased risk of liver cancer in humans, the mechanism of HCC is unknown at this time. A great deal of attention has been focused on predicting which cellular targets (e.g., "stem-cell" or mature hepatocyte) are associated with HCC as well as on phenotypic markers in HCC that can provide insight not only into MOA and origin of tumor, but also for prediction of clinical course. Examination of pathways and epigenetic changes associated with cancer, and the relationship of these changes to liver cancer are also discussed below. The field of cancer research has been transformed by the recent discoveries of epigenetic changes and their role in cancer and chronic disease states. The following discussion describes these advances but also the issues involved with the technologies that have emerged to describe them (see Section E.3.1.2, below). Exposure to TCE and its metabolites, like many others, induces a heterogeneous response, even in a relatively homogeneous genetic paradigm as the experimental laboratory rodent model. The importance of phenotypic anchoring is a major issue in the study of any MOAs using these new technologies of gene expression pattern. Although a large amount of information is now available using microarray technologies and transgenic mouse models, specifically for TCE and in study of suggested MOAs for TCE and its metabolites, use of these approaches has limitations that need to be considered in the interpretation of data and conclusions derived from such data, especially quantitative conclusions.

For TCE and its metabolites, the extent of acute to subchronic induction of hepatomegaly correlated with hepatocellular carcinogenicity, although each had differing factors contributing to that hepatomegaly from periportal glycogen deposition to hepatocellular hypertrophy and increased polyploidy. The extent of transient DNA synthesis, peroxisome proliferation, or cytotoxicity was not correlated with carcinogenicity. Hepatomegaly is also a predictor of carcinogenicity for a number of other compounds in mice and rats. Allen et al. (2004) examined the NTP database (87 compounds for rat and 83 for mice) and tried to correlate specific hepatocellular pathology in prechronic studies with carcinogenic endpoints in the chronic 2-year assays. The best single predictor of liver cancer in mice was hepatocellular hypertrophy. Hepatocellular cytomegaly and hepatocyte necrosis also contributed, although the numbers of positive findings were less than hypertrophy. With regard to genotoxicity studies, there was no evidence of a correlation between mouse liver tumor chemicals and *Salmonella* or micronucleus assay outcome. None of the prechronic liver lesions examined were correlated with either *Salmonella* or Micronucleus assays. In rats no single prechronic liver lesions (when considered individually) was a strong predictor of liver cancer in rats. The most predictive lesions was hepatocellular hypertrophy. There was not significant correlation between liver tumors/toxicity and the 2 mutagenicity measures. Although the lack of correlation with the mutagenicity assays could be interpreted as rodent assays predominantly identifying nongenotoxic liver carcinogens, this conclusion could be questioned because it is solely dependent on *Salmonella* mutagenicity and additional genotoxic endpoints could conceivably shift the association between liver cancer and genotoxicity towards a more positive correlation. As to questions of the usefulness of the mouse bioassay, the two mutagenicity assays did not correlate with rat results either and an important indicator for carcinogenicity would be lost.

Examination of tumor phenotype from TCE, DCA and TCA exposures in mice shows a large heterogeneity, which is also consistent with the heterogeneity observed in human HCC (see Section E.3.1.8, below). The heterogeneity of tumor phenotype has been correlated with survival outcome and tumor aggressiveness in humans and in transgenic mouse models that share some of the same perturbations in gene pathway expression (see Sections E.3.1.8 and E.3.2.1, below). An examination of common pathway disturbances that may be common to all cancers and those of liver tumors shows that there are pathways in common, but that there is greater heterogeneity in disturbance of hepatic pathways in cancer that may make is useful as a marker of disturbances indicative of different targets of carcinogenicity depending on the cellular context and target.

Thus, although primate and human liver may not be as susceptible to HCC as the rodent liver, the pathways leading to HCC in rodents and humans appear to be similar and heterogeneous, with some indicative of other susceptible cellular targets for neoplasia in a differing context.

E.3.1. State of Science for Cancer and Specifically Human Liver Cancer

E.3.1.1. *Epigenetics and Disease States (Transgenerational Effects, Effects of Aging and Background Changes)*

Recently, Wood et al. (2007) published their work on "genomic landscapes" of human breast and colorectal cancers that significantly forwards the understanding of "key events" involved with induction of cancer. They state that there are ~80 DNA mutations that alter amino acid in a typical cancer but that examination of the overall distribution these mutations in different cancers of the same type leads to a new view of cancer genome landscapes: they are composed of a handful of commonly mutated genes "mountains" but are dominated by a much larger number of infrequently mutated gene "hills."

> Statistical analyses suggested that most of the ~ 80 mutation in an individual tumor were harmless and that <15 were likely to be responsible for driving the initiation, progression, or maintenance of the tumor…Historically the focus of cancer research has been on the gene mountains, in part because they were the only alterations that could be identified with available technologies. However, our data show that vast majority of mutations in cancers do not occur in such mountains. This new view of cancer is consistent with the idea that a large number of mutations, each associated with a small fitness advantage, drive tumor progression. It is the "hills" and not the "mountains" that dominate the cancer genomic landscape.

The large number of "hills" actually reflects alterations in a much smaller number of cell signaling pathways. Indeed, pathways rather than individual genes appear to govern the course of tumorigenesis.

> It is becoming increasingly clear that pathways rather than individual genes govern the course of tumorigenesis. Mutations in any of several genes of a single pathway can thereby cause equivalent increases in net cell proliferation.…This new view of cancer is consistent with the idea that a large number of mutations, each associated with a small fitness advantage, drive tumor progression.

Thus, when pathways are altered the same phenotype can arise from alterations in any of several genes.

Consistent with the arguments put forth by Wood et al. (2007) for mutations in cancer is the additional insight into pathway alterations by epigenomic mechanisms, which can act similarly as mutation. Weidman et al. (2007) report that

> cell phenotype is not only dependent on its genotype but also on its unique epigenotype, which is shaped by developmental history and environmental exposures. The human and mouse genome projects identified approximately 15,500 and 29,000 CpG islands, respectively. Hypermethylation of CpG-rich regions of gene promoters inhibit expression by blocking the initiation of transcription. DNA methylation is also involved in the allelic inactivation of imprinted genes, the silencing of genes on the inactive X chromosome, and the reduction of expression of transposable elements. Because epigenomic modifications are copied after DNA synthesis by DNMT1, they are inherited during somatic cell replication…Inherited and spontaneous or environmentally induced epigenetic alterations are increasingly being recognized as early molecular events in cancer formation. Furthermore, such epigenetic alterations are potentially more adverse than nucleotide mutations because their effects on regional chromatin structure can spread, thereby affecting multiple genetic loci. Although tumor suppressor gene silencing by DNA methylation occurs frequently in cancer, genome-wide hypomethylation is one of the earliest events to occur in the genesis of cancer. Demethylation of the genome can lead to the reactivation of transposable elements, thereby altering the transcription of adjacent genes, the activation of oncogenes such as H-Ras, and biallelic expression of imprinted loci (e.g., loss of IGF2 imprinting).

Thus, epigenetic modification may be worse than mutation in terms of cancer induction.

Dolinoy et al. (2007) report on the role of environmental exposures on the epigenome, especially during critical periods of development and their role in adult disease susceptibility. They report that

> aberrant epigenetic gene regulation has been proposed as a mechanism of action for nongenotoxic carcinogenesis, imprinting disorders, and complex disorders including Alzheimer's disease, schizophrenia, asthma, and autism. Epigenetic modifications are inherited not only during mitosis but also can be transmitted transgenerationally (Rakyan et al., 2002; Rakyan et al., 2003; Anway et al., 2005). The influence on environmental factors on epigenetic gene regulation may also persist transgenerationally despite lack of continued exposure in second, third, and fourth generations (Anway et al., 2005). Therefore if the genome is compared to the hardware in a computer, the epigenome is the software that directs the computer's operation…The epigenome is particularly susceptible to deregulation during gestation, neonatal development, puberty and old age. Nevertheless, it is most vulnerable to environmental factors during embryogenesis because DNA synthetic rate is high, and the elaborate DNA methylation pattern

> and chromatin structure required for normal tissue development is established during early development…83 imprinted genes have been identified in mice and humans with 29 or about one third being imprinted in both species. Since imprinted genes are functionally haploid, they are denied the protection from recessive mutations that diploidy would normally afford. Imprinted genes that have been linked to carcinogenesis include IGF2 (bladder, lung, ovarian and others), IGF2R (breast, colon, lung, and others), and Neuronatin (pediatric leukemia).

Bjornsson et al. (2008) recently reported that not only were there time-dependent changes in global DNA methylation within the same individuals in 2 separate populations in widely separated geographic locations, these changes showed familial clustering in both increased and decreased methylation. These results were not only suggested to support the relationship of age-related loss of normal epigenetic patterns as a mechanism for late onset of common human diseases but also that losses and gains of DNA methylation observed over time in different individuals could contribute to disease with the example provided of cancer which is associated with both hypomethylation and hypermethylation through activation of oncogenes and silencing of tumor suppressor genes. The study also showed considerable interindividual age variation, with differences accruing over time within individuals that would be missed by studies that employed group averaging.

The review by Reamone-Buettner and Borlak (2007) provide insight into the role of noncoding RNAs in diseases such as cancer. They report that

> a large number of noncoding RNAs (ncRNAs) play important role in regulating gene expressions, and advances in the identification and function of eukaryotic ncRNAs, e.g., microRNAs and their function in chromatin organization, gene expression, disease etiology have been recently reviewed. The regulatory pathways mediated by small RNAs are usually collectively referred to as RNA interference (RNAi) or RNA-mediated silencing. RNAi can be triggered by small double-stranded RNA (dsRNA) either introduced exogenously into cells as small interfering siRNAs or that have been produced endogenously from small non-coding RNAs known as microRNAs (miRNAs). The dsRNAs are characteristically cleaved by the ribonuclease III-enzyme Dicer into 21- to 23 nt duplexes and the resulting fragments base-pair with complementary mRNA to target cleavage or to repress translation…Two mechanisms exist of miRNA-mediated gene regulation, degradation of the target mRNA, and translational repression. Whether one or the other of these mechanisms is used depends on the degree of the complementary between the miRNA and target mRNA. For a near perfect match, the Argonaute protein in the RNA-induced silencing complex (RISC) cleaves the mRNA target, which is destined for subsequent degradation by ribonucleases. In the situation of a less degree of complimentarity, commonly

> occurring in humans, the translational repression mechanism is used to control gene expression. However, the exact mechanism for translational inhibition is unclear.

The varying degrees in complimentarity would help explain the large number of genes that could be affected by miRNA and pleiotropic response.

The review by Feinberg et al. (2006) specifically addresses the epigenetic progenitor origin of human cancer. They conclude that epigenetic alterations are ubiquitous and serve as surrogate alterations for genetic change (oncogene activation, tumor-suppressor-gene silencing), by mimicking the effect of genetic change. They report that:

> Advances in characterizing epigenetic alterations in cancer include global alterations, such as hypomethylation of DNA and hypoacetylation of chromatin, as well as gene-specific hypomethylation and hypermethylation. Global DNA hypomethylation leads to chromosomal instability and increased tumour frequency, which has been shown *in vitro* and *in vivo* in mouse models, as well as gene-specific oncogene activation, such as R-ras in gastric cancer, and cyclin D2 and maspin in pancreatic cancer. In addition, the silencing of tumour-suppressor genes is associated with promoter DNA hypermethylation and chromatin hypoacetylation, which affect divergent genes such as retinoblastoma 1 (RB1), p16 (also known as cyclin-dependent kinase inhibitor 2A (CDKN2A), von Hippel-Lindau tumor suppressor (VHL), and MutL protein homologue (MLH1).
>
> Genetic mechanisms are not the only path to gene disruption in cancer. Pathological epigenetic changes - non-sequence-based alteration that are inherited through cell division - are increasingly being considered as alternatives to mutations and chromosomal alterations in disrupting gene function. These include global DNA hypomethylation, hypermethylation and hypomethylation of specific genes, chromatin alterations and loss of imprinting. All of these can lead to aberrant activation of growth-promoting genes and aberrant silencing of tumour-suppressor genes.
>
> Most CG dinucleotides are methylated on cytosine residues in vertebrate genomes. CG methylation is heritable, because after DNA replication the DNA methyltransferase 1, DNMT1, methylates unmethylated CG on the base-paired strand. CG dinucleotides within promoters within promoters tend to be protected from methylation. Although individual genes vary in hypomethylation, all tumours have shown global reduction of DNA methylation. This is a striking feature of neoplasia.
>
> In addition to global hypomethylation, promoters of individual genes show increased DNA methylation levels. Hypermethylation of tumour-suppressor genes can be tumour-type specific. An increasing number of genes are found to

be normally methylated at promoters but hypomethylated and activated in the corresponding tumours. These include R-RAs in gastric cancer, melanoma antigen family A, 1(MAGE1) in melanoma, maspin in gastric cancer, S100A4 in colon cancer, and various genes in pancreatic cancer.

Our genetic material is complexed with proteins in the form of histones in a one-to-one weight ratio. Core histones H2A, H2B, H3 and H4 form nucleosome particles that package 147 bp of DNA, and the linker histone H1 packages more DNA between core particles, forming chromatin. It is chromatin and not just DNA, that is the substrate for all processes that affect genes and chromosomes. In recent years, it has become increasingly evident that chromatin, like DNA methylation, can impart memory to genetic activity. There are dozens of post-translational histone modifications. Studies in many model systems have shown that particular histone modifications are enriched at sites of active chromatin (histone H3 and H4 hyperacetylation, lysing at 4 and H3 (H3-K4) dimethylation and trimethylation, and H3-K79 methylation) and others are enriched at sites of silent chromatin (H3-K9 and H3-K27 methylation). These and other histone modifications survive mitosis and have been implicated in chromatin memory.

Overproduction of key histone methyltransferases that catalyze the methylation of either H3-K4 or H3-K27 residues are frequent events in neoplasia. Global reductions in monoacetylated H4-K16 and trimethylated H4-K20 are general features of cancer cells.

Genomic imprinting is parent-of –origin-specific gene silencing. It results from a germ-line mark that causes reduced or absent expression of a specific allele of a gene in somatic cells of the offspring. Imprinting is a feature of all mammals affecting genes that regulate cell growth, behaviour, signaling, cell cycle and transport; moreover, imprinting is necessary for normal development. Imprinting is important in neoplasia because both gynogenotes (embryos derived only from the maternal genetic complement) and androgenotes (embryos derived only from the paternal genetic complement) form tumours – ovarian teratomas, and hydtidiform moles/ choriocarcinomas, respectively. Loss of imprinting (LOI) refers to activation of the normally silenced allele, or silencing of the normally active allele, of an imprinted gene. LOI of the insulin-like growth factor 2 gene (IGF2) accounts for half of Wilms tumours in children. LOI of IGF2 is also a common epigenetic variant in adults and is associated with a fivefold increased frequency of colorectal neoplasia. LOI of IGF2 might cause cancer by increasing the progenitor cell population in the kidney in Wilm's tumor and in the gastrointestinal tract in colorectal cancer.

Feinberg et al. (2006) propose that epigenetic changes can provide mechanistic unity to understanding cancer, they can occur earlier and set the stage for genetic alterations, and have been linked to the pluripotent precursor cells from which cancers arise. "To integrate the idea of these early epigenetic events, we propose that cancer arises in three steps; an epigenetic disruption of progenitor cells, an initiating mutation and genetic and epigenetic plasticity."

> The first step involves an epigenetic disruption of progenitor cells in a given organ or system, which leads to a polyclonal precursor population of neoplasia-ready cells. These cells represent a main target of environmental, genetic and age-dependent exposure that largely accounts for the long latency period of cancer. Epigenetic disruption might perturb the normal balance between undifferentiated progenitor cells and differentiated committed cells within a given anatomical compartment, either in number or in their capacity for aberrant differentiation, which provides a common mechanism of neoplasia.
>
> All tumours show global changes in DNA methylation, and DNA methylation is clonally inherited through cell division. Because the conventional genetic changes in cancer are also clonal, global hypomethylation would have to occur universally, at the same moment as the mutational changes, which seems unlikely. This suggests that global DNA hypomethylation (and global reductions of specific histone modifications) precedes genetic change in cancer. Similarly, hypermethylation of tumour-suppressor genes has been observed in the normal tissue of patients in which the same gene is hypermethylated in the tumour tissue. Recent data demonstrate LOI of IGF2 throughout the normal colonic epithelium of patients who have LOI-associated colorectal cancer. LOI is associated with increased risk of intestinal cancers in both humans and mice. A specific change in the epithelium is seen in mice that are engineered to have biallelic expression of IGF2 – a shift in the proportion of progenitor to differentiated cells throughout the epithelium; a similar abnormality was observed in humans with LOI of IGF2.
>
> The proposed existence of the epigenetically disrupted progenitors of cancer implies that the earliest stages in neoplastic progression occur even before what a pathologist would recognize as a benign pre-neoplastic lesion. Such alterations are inherently polyclonal. This is in contrast with the widely accepted model of cancer as a monoclonal disorder that arises from an initiating mutation- a model that was proposed and accepted when little was known about epigenetic phenomena in cancer.

Thus, Feinberg et al. (2006) provide a hypothesis for the latency period of cancer and suggest that epigenetic changes predate mutational ones in cancer. Tissues that look phenotypically "normal" may harbor epigenetic changes and predispositions toward neoplasia. In regard to what cells may be targets or epigenetic changes that can be "progenitor cells" in the case of cancer, Feinberg et al. (2006) define such cell having "capacity for self-renewal and pluripotency – over their tendency toward limited replicative potential and differentiation." Within the liver, there are multiple cell types that would fit such a definition including those who are considered "mature" (see Section E.3.1.4, below). Feinberg et al. (2006) also note that epigenetic states can be continuously modified to become heterogeneous at all states of the neoplastic process.

> Telomere erosion results in chromosome shortening and uncapped ends that begin to fuse and the resulting dicentric chromosomes break at anaphase. DNA palindromes have recently been found to form at high levels in cancer cells. Like telomere erosion, DNA palindrome formation can lead to genetic instability by initiating bridge-breakage-fusion cycles. However, it is not known how or exactly when palindromes form, although they appear early in cancer progression. Epigenetic instability can also promote cancer through pleiotropic alterations in the expression of genes that modify chromatin.
>
> Epigenetic changes are reversible but the changes can initiate irreversible genetic changes. Permanent epigenetic changes can have an epigenetic basis. On a background of cancer-associated epigenetic instability, the effects of mutations in oncogenes and tumour –suppressor genes might be exacerbated. Therefore the risk of developing malignancy would be much higher for a given mutations event if it occurred on the background of epigenetic disruption.
>
> The environmental dependence of cancer fits an epigenetic model generally for human disease – the environment might influence disease onset not simply through mutational mechanisms but in epigenetically modifying genes that are targets for either germline or acquired mutation; that is, by allowing genetic variates to be expressed. Little is known about epigenetic predispositions to cancer, but a recent twin study indicates that, similar to cancer risk, global epigenetic changes show striking increase with age.
>
> Environmental insults might affect the expression of tumour-progenitor genes, leading to both genetic and epigenetic alterations. Liver regeneration after tissue injury leads to widespread hypomethylation and hypermethylation of individual genes; both of these epigenetic changes occur in cancer.

In regard to the implications of epigenomic changes and human susceptibility to toxic insult, the review by Szyf (2007) provides additional insights.

> The basic supposition in the field has been that the interindividual variations in response to xenobiotic are defined by genetic differences and that the main hazard anticipated at the genomic level from xenobiotic is mutagenesis or physical damage to DNA. In accordance with this basic hypothesis, the main focus of attention in pharmacogenetics has been on identifying polymorphisms in genes encoding drug metabolizing enzymes and receptors. New xenobiotics were traditionally tested for their genotoxic effects. However, it is becoming clear that epigenetic programming plays an equally important role in generating interindividual phenotypic differences, which could affect drug response.

> Moreover, the emerging notion of the dynamic nature of the epigenome and its responsibility to multiple cellular signaling pathways suggest that it is potentially vulnerable to the effects of xenobiotics not only during critical period in development but also later in life as well. Thus, non-genotoxic agents might affect gene function through epigenetic mechanisms in a stable and long-term fashion with consequences, which might be indistinguishable form the effects of physical damage to the DNA. Epigenetic programming has the potential to persist and even being transgenerationally transmitted (Anway et al., 2005) and this possibility creates a special challenge for toxicological assessment of safety of xenobiotics. Any analysis of interindividual phenotype diversity should therefore take into account epigenetic variations in addition to genetic sequence polymorphisms. Whereas, a germ-line polymorphism is a static property of an individual and might be mapped in any tissue at any point in life, epigenetic differences must be examined at different time points and at diverse cell types.

Karpinets and Foy (2005) propose that epigenetic alterations precede mutations and that succeeding mutations are not random but in response to specific types of epigenetic changes the environment has encouraged. This mechanism was also suggested as to both explain the delayed effects of toxicant exposure and the bystander effect of radiation on tumor development, which are inconsistent with the accepted mechanism of direct DNA damage.

> In a study of ionizing radiation, non-irradiated cells acquired mutagenesis through direct contact with cells whose nuclei had previously been irradiated with alpha-particles (Zhou et al., 2003). Molecular mechanisms underlying these experimental findings are not known but it is believed that it may be a consequence of bystander interactions involving intercellular signaling and production of cytokines (Lorimore et al., 2003).

Caldwell and Keshava (2006) report that

> aberrant DNA methylation has emerged in recent years as a common hallmark of all types of cancers with hypermethylation of the promoter region of specific tumor suppressor genes and DNA repair genes leading to their silencing (an effect similar to their mutation), and genomic hypomethylation (Ballestar and Esteller, 2002; Berger and Daxenbichler, 2002; Herman et al.,1998; Pereira et al. 2004; Rhee et al., 2002). Whether DNA methylation is a consequence or cause of cancer is a long-standing issue (Ballestar and Esteller, 2002). Fraga et al. (2004, 2005) report global loss of monoacetylation and trimethylation of histone H4 as common a hallmark of human tumor cells but suggest genomone-wide loss of 5-methylcytosine (associated with the acquisition of a transformed phenotype) does not exist as a static predefined value throughout the process of carcinogenesis but as a dynamic parameter (i.e., decreases are seen early and become more marked in later stages).

E.3.1.2. *Emerging Technologies, DNA and siRNA, miRNA Microarrays—Promise and Limitations for Modes of Action (MOAs)*

Currently new approaches are emerging for the study of changes in gene expression and protein production induced by chemical exposure that could be related to their toxicity and serve as an anchor for determining similar patterns between rodent models and human diseases or risks of chemically-induced health impacts. Such approaches have the promise to extend the definitions of "genotoxic" and "nongenotoxic" effects which with the advent of epigenomic study have become obsolete as they assume only alteration of the DNA sequence is important in cancer induction and progression. However, not only is phenotypic anchoring an issue in regard to the differing cell types, regions, and lobes of the liver (see Section E.1.2, above), it is also an issue for overall variability of response between animals and is critical for interpretation of microarray and other genomic database approaches. As shown in the discussions of TCE effects in animal models, TCE treatment resulted in a large variability in response between what are supposed to be relatively homogeneous genetically similar animals and there was an apparent difference in response between studies using the same paradigm. It is important that as varying microarray approaches and analyses of TCE toxicity or of potential MOAs are published, the issue of phenotypic anchoring at the cellular to animal level is addressed. Several studies of TCE microarray results and those of PPARα agonists have been reported in the literature in an attempt to discern MOAs. Issues related to conduct of these experiments and interpretation of their results are listed below.

Perhaps one of the most important studies of this issue has been reported by Baker et al. (2004). The ILSI HESI formed a hepatotoxicity working group to evaluate and compare biological and gene expression responses in rats exposed to well-studied hepatotoxins (Clofibrate and methapyrilene), using standard experimental protocol and to address the following issues: (a) how comparable are the biological and gene expression data from different laboratories running identical *in vivo* studies (b) how reproducible are the data generated across laboratories using the same microarray platform (c) how do data compare using different microarray platforms; (d) how do data compare using RNA from pooled and individual animals; (e) do the gene expression changes demonstrate time- and dose-dependent responses that correlate with known biological markers of toxicity? (Baker et al., 2004). The rat model studied was the male S-D rat (57 or 60−66 days of age) exposed to 250 or 25 mg/kg/d Clofibrate for 1, 3 or 7 days. Two separate *in vivo* studies were conducted: one at Abbott Laboratories and on at GlaxoSmithKline (GSK, in United Kingdom [UK]). There was a difference in biological response between the two laboratories. The high dose (250 mg/kg/d) group at Day 3 had a 15% increase in liver weight relative to body weight in the GSK study, compared with a 3% liver weight increase in the

Abbott study. At 7-days, there was a 31% liver weight increase in the GSK study and 15% in the Abbott study. Observed changes in clinical chemistry parameters also indicated difference in the biological response of the *in vivo* study concordant with difference in liver weight. A significant reduction in total cholesterol levels was seen in the GSK study at the high dose for all time points. However, the Abbott study demonstrated a significant reduction only at one dose and time point. The incidence of mitotic figures also differed between the labs. In both studies there was a 2−3 times greater Acyl-CoA enzyme (ACOX) activity at the high dose but no difference from control in the low dose. Again the GSK lab gave greater response. For microarrays, GSK and ULR pooled samples from each treatment group of four animals. U.S. EPA did some of the microarray analyses as well as GSK and ULR (GSK in UK). It is apparent that although the changes in genes were demonstrated by both laboratories, there were quantitative differences in the fold change values observed between the two sites.

The U.S. EPA analyzed gene expression in individual RNA samples obtained from Day 7 high and low-dose animals that had been treated at Abbot. GSK (U.S.) and ULR analyzed gene expression in pooled RNA from Day 7 high and low dose animals treated at GSK (UK). Gene expression data from individual animal samples indicated that 7 genes were significantly upregulated (maximum of 7.2-fold) and 12 were down regulated (maximum of 4.3-fold decrease) in the high-dose group. The low-dose group generated only one statistically significant gene expression change, namely heat shock protein 70 (HSP70). In comparison, expression changes in the 7-day pooled high-dose samples analyzed by GSK (U.S.) ranged from 43.3-fold to a 3.5-fold decrease. Changes in these same samples analyzed by ULR ranged from a 4.9-fold increase to a 4.3-fold decrease. As an example, the microarray fold change at 7-day 250 mg/kg/d Clofibrate showed a 3.8-fold increase for U.S. EPA individual animals sampled, and 2.2-fold increase for pooled samples by ULR, and a 20.3-fold increase in pooled samples by GSK (U.S.) for CYP4A1 (Baker et al., 2004). Thus, these results show a very large difference not only between treatment groups but between pooled an nonpooled data and between labs analyzing the same RNA.

Not only was there a difference in DNA microarray results but a comparison of gene expression data from Day 7 high-dose samples obtained using quantitative realtime PCR versus data generated using cDNA microarrays has shown a quantitative difference but qualitative similar patterns. Although both methods of quantitative real time PCR on the pooled sample showed the PPARα gene to be down regulated, the GSK (U.S.) pooled sample microarray analysis indicated upregulation; the URL pooled and U.S. EPA individual microarray analyses showed no change. The microarray for PPARα at 7-day 250 mg/kg/d Clofibrate showed no change for individual animals (U.S. EPA), no change for pooled samples (ULR) and upregulation of 1.8-fold value for pooled samples for GSK(U.S.). The quantitative real time PCR on the pooled sample using Taqman gave a 4.5-fold down regulation and using SYBR Green gave a 1.2-fold down regulation of PPARα.

Baker et al. (2004) reported that the pooling of samples for microarray analysis has been used in the past to defray the cost of microarray experiments, reduce the effect of biological variation, and in some cases overcome availability of limiting amounts of tissues. Unfortunately this approach essentially produced a sample size (*n*) of one animal. Repeated microarray experiments with such pooled RNA produces technical replicates as opposed to true biological replicates and thus, does not allow calculation of biologically significant changes in gene expression between different dose groups or time points. Another possible consequence of pooling is to mask individual gene changes and leave open the possibility of introducing error due to individual outlier responses.

Woods et al. (2007a) note that

> because toxicogenomics is a relatively novel technology, there are a number of limitations that must be resolved before array data is widely accepted. Microarray studies have been touted as being highly sensitive for detecting toxic responses at much earlier time points and/or lower doses than histopathology, clinical chemistry or other traditional toxicological assays can detect. However, based on the nature of the assay, measurements of extreme levels of gene expression – low or high –are thought to be unreliable. Also the reproducibility of microarray experiments has raised concerns. “Batch effects” based on the day, user, and laboratory environment have been observed in array datasets. To address these concerns, confirmation of microarray-derived gene expression profiles is typically performed using quantitative real time polymerase chain reaction (RT-PCR) or Northern blot analysis.

In addition to the issues raised above, Waxman and Wurmbach (2007) raise issues regarding how quantitative realtime PCR experiments are conducted. They state that cancer development affects almost all pathways and genes including the “housekeeping” genes, which are involved in the cell’s common basic functions (e.g., glyceraldehyde-3-phosphate dehydrogenase [GADPH], beta actin [ACTB], TATA-binding protein, ribosomal proteins, and many more). However, “many of these genes are often used to normalize quantitative real-time RT-PCR (qPCR) data to account for experimental differences, such as differences in RNA quantity and quality, the overall transcriptional activity and differences in cDNA synthesis. GADPH and ACTB are most commonly used for normalization, including studies of cancer.” Waxman and Wurmbach (2007) suggest that despite the fact that it has been shown that these genes are differentially expressed in cancers, including colorectal-, prostate-, and bladder-cancer, some qPCR studies on hepatocellular carcinoma used GAPDH or ACTB for normalization. Since many investigations on cancer include multiple comparisons, and analyze different stages of the disease, such as normal tissue, preneoplasm, and consecutive stages of cancer, "it crucial to find an appropriate gene for normalization" whose expression is constant throughout all disease stage and not response to treatment. For liver cancers associated with exposure to hepatitis C virus (HCV), Waxman and Wurmbach (2007) reported that differing states, including preneoplastic lesions (cirrhosis and dysplasia) and consecutive stages of hepatocellular carcinoma, had differential expression of "housekeeping" genes and that using them for normalization had an effect on the fold change of qPCR data and on the general direction (up or down) of differentially expressed genes. For example, GAPDH was strongly upregulated in advanced and very advanced stages of hepatocellular carcinoma (in some samples up to 7-fold) and ACTB was up-regulated 2- to 3-fold in many advanced and very advanced tumor samples. Waxman and Wurmbach (2007) conclude that

> microarray data are known to be highly variable. Due to its higher dynamic range qPCR is thought to be more accurate and therefore is often used to corroborate microarray results. Mostly, general direction (up and down-regulation) and rank order of the fold-changes are similar, but the levels of the fold changes of microarray experiments differ compared to qPCR data and show a marked tendency of being smaller. This effect is more pronounced as the fold change is very high.

In relation to use of gene expression and indicators of cancer causation, Volgelstein and Kinzler (2004) make important points regarding their use:

> Levels of gene expression are unreliable indicators of causation because disturbance of any network invariably leads to a multitude of such changes only peripherally related to the phenotype. Without better ways to determine whether an unmutated but interesting candidate gene has a causal role in neoplasia, cancer researchers will likely be spending precious time working on genes only peripherally related to the disease they wish to study.

This is important caveat for gene expression studies for MOA that are "snapshots in time" without phenotypic anchoring and even more applicable to experimental paradigms where there is ongoing necrosis or toxicity in addition to gene changes that may or may not be associated with neoplasia.

For an endpoint that is not as complex as neoplasia, there are issues regarding uses of microarray data. In regard to the determination of acute liver toxicity caused by one of the most studied hepatotoxins, acetaminophen, and its correlation with microarray data, Beyer et al. (2007) also have reported the results of a landmark study examining issues regarding use of this approach.

> The biology of liver and other tissues in normal and disease states increasingly is being probed using global approaches such as microarray transcriptional profiling. Acceptance of this technology is based principally on a satisfactory level of reproducibility of data among laboratories and across platforms. The issue of reproducibility and reliability of genomics data obtained from similar (standardized) biological experiments performed in different laboratories is crucial to the generation and utility of large databases of microarray results. While several recent studies uncovered important limitation of expression profiling of chemical injury to cells and tissues (Baker et al 2004; Beekman et al 2006; Ulrich et al 2004), determining the effects of intralaboratory variables on the reproducibility, validity, and general applicability of the results that are generated by different laboratories and deposited into publicly available databases remains a gap…The National Institutes of Environmental Health Sciences (NIEHS) established the Toxicogenomics Research Consortium to apply the collective and specialized expertise from academic institutions to address issues in integrating gene expression profiling, bioinformatics, and general toxicology. Key elements include developing standardized practices for gene expression studies and conducting systematic assessments of the reproducibility of traditional toxicity endpoints and microarray data within and among laboratories. To this end the consortium selected the classical hepatotoxicant acetaminophen (APAP) for its proof of concept experiments. Despite more than 30 years of research on APAP, we are far from a complete understanding of the mechanisms of liver injury, risk factors, and molecular markers that predict clinical outcome after poisoning. APAP–induced hepatotoxicity was performed at seven geographically dispersed Centers. Parallel studies with N-acetyl-m-aminophenol (AMAP), the non-hepatotoxic isomer of APAP, provided a method to isolate transcripts associated with hepatotoxicity (Beyer et al., 2007).

Beyer et al identified potential sources of interlaboratory variability when microarray analyses were conducted by one laboratory on RNA samples generated in different laboratories but using the same experimental paradigm and source of animals. Toxic injury by APAP showed variability across Centers and between animals (e.g., percent liver affected by necrosis [<20 to 80% at one time period and 0 to 60% at another], control animal serum ALT [3-fold difference], and in glutathione depletion [<5 to >60%] between centers). There was concordance between APAP toxicity as measured in individual animals (rather than expressed as just a mean with SE) and transcriptional response. Of course the variability between gene platforms and processing of the microarrays had been reduced by using the same facility to do all of the microarray analyses. However, the results show that phenotypic anchoring of gene expression data are required for biologically meaningful meta-analysis of genomic experiments.

Woods et al. (2007a) note that

> improvements should continue to be made on statistical analysis and presentation of microarray data such that it is easy to interpret. Prior to the current advances in bioinformatics, the most common way of reporting results of microarray studies involved listing differentially expressed genes, with little information about the statistical significance or biological pathways with which the genes are associated.

However, there are issues with the use of "Classifiers" or predictive genomic computer programs based on genes showing altered expression in association with the observed toxicities.

> Although these metrics built on different machine learning algorithms could be useful in estimating the severity of potential toxicities induced by compounds, the applications of these classifiers in understanding the mechanisms of drug-induced toxicity are not straightforward. In particular this approach is unlikely to distinguish the upstream causal genes from the downstream responsive genes among all the genes associated with an induced toxicity. Without knowledge of the causal sufficiency order, designing experiments to test predicted toxicity in animal models remains difficult" (Dai et al., 2007).

Ulrich (2003) states limitation of microarray analysis to study nuclear receptors (e.g., PPARα).

> Nuclear receptors comprise a large group of ligand-activated transcription factors that control much of cellular metabolism. Toxicogenomics is the study of the structure and output of the entire genome as it related and responds to adverse xenobiotic exposure. Traditionally, the genes regulated by nuclear receptors in cells exposed to toxins have been explored at the mRNA and protein levels using northern and western blotting techniques. Though effective when studying the expression of individual genes, these approaches do not enable the understanding of the myriad of genes regulated by individual receptors or of the crosstalk between receptors…Discovery of the multiple genes regulated by each receptor type has thus been driven by technological advances in gene expressional analysis, most commonly including differential display, RT-PCR and DNA microarrays., and in the development or receptor transgenic and knockout animal models. There is much cross talk between receptors and many agonists interact with multiple receptors. Off target effects cannot be predicted by target specificity. Though RCR can affect transcription directly, much of its effects are

exerted through heterodimeric binging with other nuclear receptors (PXR, CAR, PPARα, PPARγ, FXR, LXR, TR) (Ulrich, 2003).

Another tool recent developed is gene silencing by introduction of siRNA. Dai et al. (2007) note issues involved in the siRNA to change gene expression for exploration of MOA etc. to include the potential of off-target effects, incomplete knockdown, and nontargeting of splice variants by the selected siRNA sequence. Using knockdown of PPARα in mice, Dai et al. (2007) report "PPARα knockdown was variable between mice ranging from ~ 80 % knockdown to little or no knockdown and that differing siRNAs gave different patterns of gene expression with some grouped with PPARα -/- null mice but others grouped with expression patterns of mice injected with control siRNA or Ringers buffer alone and showing no PPARα knockdown." Dai et al concluded that it is possible that it is the change in PPARα levels that is important for perturbing expression of genes modulated by PPARα rather than the absolute levels of PPARα. Not only is the finding of variability in knockdowns by siRNA technologies important but The finding that level of PPAR is not necessarily correlated with function and that it could be the change and not absolute level that matters in modulation in gene expression by PPARα is of importance as well. How an animal responds to decreased PPARα function may also depend on its gender. Dai et al. (2007) observed more dramatic phenotypes in female vs. male mice treated with siRNA and noted that in aged PPARα -/- mice, Costet et al. (1998) have reported sexually dimorphic phenotypes including obesity and increased serum triglyceride levels in females, and steatosis and increased hepatic triglyceride levels in males.

In regard to the emerging science and preliminary reports of the effects of microRNA as oncogenes and tumor suppressors and of possible importance to hypothesized MOAs for liver cancer, the same caveats as described for DNA microarray analyses all apply along with additional uncertainties. miRNAs repress their targeted mRNAs by complementary base pairing and induction of the RNA interference pathway. Zhang et al. (2007) report Northern blot detection of gene expression at the mRNA level and its correlation with miRNA expression in cancer cells as well as realtime PCR. These PCR-based analyses quantify miRNA precursors and not the active mature miRNAs. However, they report that the relationship between pri-miRNA and mature miRNA expression has not been thoroughly addressed and is critical in order to use real time PCR analysis to study the function of miRNAs in cancers. They go on to state that

> although Northern Blotting is a widely used method for miRNA analysis, it has some limitations, such as unequal hybridization efficiency of individual probes and difficulty in detecting multiple miRNAs simultaneously. For cancer studies,

it is important to be able to compare the expression pattern of all known miRNAs between cancer cells and normal cells. Thus, it is better to have methods which detect all miRNA expression at a single time…Although Northern blot analysis, real-time PCR, and miRNA microarray can detect the expression of certain miRNAs and determine which miRNAs may be associated with cancer formation, it is difficult to determine whether or not miRNAs play a unique role in cancers. Also these techniques cannot directly determine the correlation between mRNA expression levels and whether the up-regulation or down-regulation of certain miRNAs is the cause of cancer or a downstream effect of the disease…Many miRNA genes have been found that are significantly overexpressed in different cancers. All of them appear to function as oncogenes; however, only a few of them have been well characterized.

Zhang et al. (2007) suggest that bioinformatic studies indicate that numerous genes are the targets of miR-17-92: more than 600 for miR-19a and miR-20, two members of the miR-17-92 cluster.

Cho (2007) state that

though more than 530 miRNAs have been identified in human, much remains to be understood about their precise cellular function and role in the development of diseases…Although each miRNA can control hundreds of target genes, it remains a great challenge to identify the accurate miRNA targets for cancer research.

Thus, miRNAs have multiple targets so, like other transcription factors, may have pleotropic effects that are cell, timing, and context specific.

Vogelstein and Kinzler (2004) state "in the last decade many important gene responsible for the genesis of various cancers have been discovered." Most importantly they and others suggest that pathways rather than individual gene expression should be the focus of study. As a specific example, Volgelstein and Kinzler note

another example of the reason for focusing on pathways rather than individual genes has been provided by studies of TP53 tumor-suppressor gene. The p53 protein is a transcription factor that normally inhibits cell growth and stimulates cell death when induced by cellular stress. The most common way to disrupt the p53 pathway is through a point mutation that inactivates its capacity to bind specifically to its cognate recognition sequence. However, there are several other ways to achieve the same effects, including amplification of the MDM2 gene and infection with DNA tumor viruses whose products bind to p53 and functionally inactivate it.

In regard to cellular anchoring for gene expression or pathway alterations associated with cancer and the importance of "context" of gene expression changes, Vogelstein and Kinzler (2004) give several examples.

> In solid tumors the important of the interactions between stroma and epithelium is becoming increasingly recognized (e.g., the importance of the endothelial cell)…One might expect that a specific mutation of a widely expressed gene would have identical or at least similar effects in different mammalian cell types. But this is not in general what is observed. Different effects of the same mutation are not only found in distinct cell types; difference can even be observed in the same cell types, depending on when the mutation occurred during the tumorigenic process. The RAS gene mutations provide informative examples of these complexities. *KRAS2 g*ene mutation in normal pancreatic duct cells seem to initiate the neoplastic process, eventually leading to the development of pancreatic cancer. The same mutations occurring in normal colonic or ovarian epithelial cells lead to self-limiting hyperplastic or borderline lesions that do not progress to malignancy. In many human and experimental cancers, *RAS* genes seem to function as oncogenes. But *RAS* genes can function as suppressor genes under other circumstances, inhibiting tumorigenesis after administration of carcinogens to mice. These and similar observation on other cancer genes are consistent with the emerging notion that signaling molecules play multiple roles at multiple time, even in the same cell type. However, the biochemical bases for such variations among cancer cells are almost unknown.

In regard to the major pathways and mediators involved in cancer several investigators have reported a coherent set that are involved in many types of cancers. Vogelstein and Kinzler (2004) note that major pathways and mediators include p53, RB, WNT, E-cadherin, GL1, APC, ERK, RAS:GTP, P13K,SMAD, RTK BAD, BAX, and H1F1. In regard to coherence and site concordance between animal and human data, the disturbance of a pathway in one species may result in the different expression of tumor pattern in another but both linked to a common endpoint of cancer. Thus, pathways rather than a single mutation should be the focus of MOA and cancer as several actions can be manifested by one pathway or change at one time that lead to cancer.

Vogelstein and Kinzler (2004) also note that pathways that are common to "cancer" are also operative in liver cancer where, as a heterogeneous disease, multiple pathways have been implicated in differing manifestations of this disease. Thus, liver cancer may be an example in its multiple forms that are analogous to differing sites being affected by common pathways leading to "cancer." Pathway concordance may not always show up as site concordance as expression of cancer between species. Liver cancer may be the example where many pathways can lead a cancer that is characterized by its heterogeneity.

E.3.1.3. *Etiology, Incidence and Risk Factors for Hepatocellular Carcinoma (HCC)*

The review article of Farazi and DePinho (2006) provides and excellent summary of the current state of human liver cancer in terms of etiology and incidence. The 5-year survival rate of individuals with liver cancer in the United States is only 8.9% despite aggressive conventional therapy with lethality of liver cancer due in part from its resistance to existing anticancer agents, a lack of biomarkers that can detect surgically respectable incipient disease, and underlying liver disease that limits the use of chemotherapeutic drugs. Chen et al. (2002) report that surgical resection is considered the only "curative treatment" but >80 of patients have widespread HCC at the time of diagnosis and are not candidates for surgical treatment. Among patients with localized HCC who undergo surgery, 50% suffer a recurrence. Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality. HCC accounts for between 85 and 90% of primary liver cancers (El-Serag and Rudolph, 2007). Seitz and Stickel (2006) report that epidemiological data from the year 2000 indicate that more than 560,000 new cases of HCC occurred worldwide, accounting for 5.6% of all human cancers and that HCC is the fifth most common malignancy in men and the eighth in women. Overall, incidence rates of HCC are higher in males compared to females. In almost all populations, males have higher liver cancer rates than females, with male:female ratios usually averaging between 2:1 and 4:1 and the largest discrepancies in rates (>4:1) found in medium-risk European populations (El-Serag and Rudolph, 2007). Experiments show a 2- to 8-fold of control HCC development in male mice as well supporting the hypothesis that androgens influence HCC progression rather than sex-specific exposure to risk factors (El-Serag and Rudolph, 2007). El-Serag and Rudolph (2007) also report that

> in almost all areas, female rates peak in the age group 5 years older than the peak age group for males. In low risk population (e.g., U.S.) the highest age-specific rates occur among persons aged 75 and older. A similar pattern is seen among most high-risk Asian populations. In contrast male rats in high-risk African populations (e.g., Gambia) ten to peak between ages 60 and 65 before declining, whereas female rates peak between 65 and 70 before declining.

Age adjusted incidence rates for HCC are extremely high in East and Southeast Asia and in Africa but in Europe, there is a gradually decreasing prevalence from South to North. HCC incidence rates also vary greatly among different populations living in the same region and vary by race (e.g., for all ages and sexes in the United States, HCC rates are 2 times higher in Asian than in African Americans, whose rates are 2 times higher than those in whites) ethnic variability likely to include differences in the prevalence and acquisition time of major risk factors for liver disease and HCC (El-Serag and Rudolph, 2007). Worldwide HCC incidence rate doubled during the last two decades and younger age groups are increasingly affected (El-Serag, 2004). The high prevalence of HCC in Asia and Africa may be associated with widespread infection with hepatitis B virus (HBV) and HCV but other risk factors include chronic alcohol misuse, non alcoholic fatty liver disease (NAFLD), tobacco, oral contraceptives, and food contamination with aflatoxins (Seitz and Stickel, 2006). El-Serag and Rudolph (2007) report HCC to be the fastest growing cause of cancer-related death in men in the United States with age-adjusted HCC incidence rates increasing more than 2-fold between 1985 and 2002 and that, overall, 15−50% of HCC patients in the United States have no established risk factors.

Although liver cirrhosis is present in a large portion of patients with HCC, it is not always present. Fattovich et al. (2004) report that

> differences of geographic area, method of recruitment of the HCC cases (medical or surgical) and the type of material studied (liver biopsy specimens, autopsy, or partial hepatectomies) may account for the variable prevalence of HCC without underlying cirrhosis (7% to 54%) quoted in a series of studies. Percutaneous liver biopsy specimens are subject to sampling error. However, only a small proportion of patients with HCC without cirrhosis have absolutely normal liver histology, the majority of them showing a range of fibrosis intensity from no fibrosis are all to septal and bridging fibrosis, necroinflammation, steatosis, and liver cell dysplasia.

Farazi and DePinho (2006) note that for diabetes, a higher indices of HCC has been described in diabetic patients with no previous history of liver disease associated with other factors. El-Serag and Rudolph (2007) report that in their study of VA patients (173,643 patients with and 650,620 patients without diabetes), that HCC incidence doubled among patients with diabetes and was higher among those with a longer follow-up of evaluation. “Although most studies have been conducted in low HCC rate areas, diabetes also has been found to be a significant risk factor in areas of high HCC incidence such as Japan. Taken together, available data suggest that diabetes is a moderately strong risk factor for HCC.”

NAFLD and nonalcoholic steatohepatitis contribute to the development of fibrosis and cirrhosis and therefore, might also contribute to HCC development. The pathogenesis of NAFLD includes the accumulation of fat in the liver which can lead to reactive oxygen species in the liver with necrosis factor α (TNFα) elevated in NAFDL and alcoholic liver disease (Seitz and Stickel, 2006). Abnormal liver enzymes not due to alcohol, viral hepatitis, or iron overload are present in 2.8 to 5.5% of the United States general population and may be due to NAFLD in 66 to 90% of cases (Adams and Lindor, 2007). Primary NAFLD occurs most commonly and is associated with insulin-resistant states, such as diabetes and obesity with other conditions associated with insulin resistance, such as polycystic ovarian syndrome and hypopituitarism also associated with NAFLD (Adams and Lindor, 2007). The steatotic liver appears to be susceptible to further hepatotoxic insults, which may lead to hepatocyte injury, inflammation, and fibrosis, but the mechanisms promoting progressive liver injury are not well defined (Adams and Lindor, 2007). Substrates derived from adipose tissue such as FFA, TNF-α, leptin, and adiponectin have been implicated with oxidative stress appearing to be important leading to subsequent lipid peroxidation, cytokine induction, and mitochondrial dysfunction. Liver disease was the third leading cause of death among NAFLD patients compared to the 13th leading cause among the general population, suggesting that liver-related mortality is responsible for a proportion of increased mortality risk among NAFLD patients (Adams and Lindor, 2007).

The relative risk for HCC in type 2 diabetics has been reported to be approximately 4 and increases to almost 10 for consumption of more than 80 g of alcohol per day (Hassan et al., 2002). El-Serag and Rudolph (2007) report that

> it has been suggested that many cryptogenic cirrhosis and HCC cases represent more severe forms of nonalcoholic fatty liver disease (NAFLD), namely nonalcoholic steato hepatitis (NASH). Studies in the United States evaluating risk factors for chronic liver disease or HCC have failed to identify HCV, HBV, or heavy alcohol intake in a large proportion of patients (30-40%). Once cirrhosis and HCC are established, it is difficult to identify pathologic features of NASH. Several clinic-based controlled studies have indicated that HCC patients with cryptogenic cirrhosis tend to have clinical and demographic features suggestive of NASH (predominance of women, diabetes, and obesity) as compared with age- and sex-matched HCC patients of well defined vial or alcoholic etiology. The most compelling evidence for an association between NASH and HCC is indirect and come from studies examining HCC risk with 2 conditions strongly associated with NASH: obesity and diabetes. In a large prospective cohort in the US, followed up for 16 years, liver cancer mortality rates were 5 times greater among men with the greatest baseline body mass index (range 35-40) compared with those with a normal body mass index. In the same study, the risk of liver cancer was not as increase in women, with a relative risk of 1.68. Two other population-based cohort studies from Sweden and Denmark found excess HCC risk (increased 2- to 3-fold) in obese men and women compared with those with a normal body mass index…Finally, liver disease occurs more frequently in those with more severe metabolic disturbances, with insulin resistance itself shown to increase as the disease progresses. Several developed countries most notably the United States, are in the midst of a burgeoning obesity epidemic. Although the evidence linking obesity to HCC is relatively scant, even small increase in risk related to obesity could translate into a large number of HCC cases.

Thus, even a small increase in risk related to obesity could result in a large number of HCC cases and the latency of HCC may make detection of increased HCC risk not detectable for several years.

Other factors are involved as not every cirrhotic liver progresses to HCC. Seitz and Stickel (2006) suggest that 90 to 100% of those who drink heavily suffer from alcoholic fatty liver, 10−35% of those evolve to alcoholic steatohepatitis, 8−20% of those evolve to alcoholic cirrhosis, and 1−2% of those develop HCC. HCV infects approximately 170 million individuals worldwide with approximately 20% of chronic HCV cases developing liver cirrhosis and 2.5% developing HCC. Infection with HBV, a noncytopathic, partially double stranded hepatotropic DNA virus classified as a member of the hepadnaviridae family, is also associated with liver cancer risk with several lines of evidence supporting the direct involvement of HBV in the transformation process (Farazi and DePinho, 2006). El-Serag and Rudolph (2007) suggest that

> Epidemiologic research has shown that the great majority of adult-onset HCC cases are sporadic and that many have at lease 1 established non-genetic risk factor such as alcohol abuse or chronic HCV or HBV infection. However, most people with these known environmental risk factors never develop cirrhosis or HCC, whereas a sizable minority of HCC case develop among individuals without any known risk factors…Genetic epidemiology studies in HCC, similar to several other conditions, have fallen short of early expectations that they rapidly and unequivocally would result in identification of genetic variants conveying substantial excess risk of disease and thereby establish the groundwork for effective genetic screening for primary prevention.

E.3.1.4. ***Issues Associated with Target Cell Identification***

Another outstanding and important question in HCC pathogenesis involves the cellular origin of this cancer. The liver is made up of a number of cell types showing different phenotypes and levels of differentiation. Which cell types are targets of hepatocarcinogens and are those responsible for human HCC is a matter of intense debate. Studies over the last decade provide evidence of several types of cells in the liver that can repopulate the hepatocyte compartment after a toxic insult. “Indeed, although the existence of a liver stem cell is often debated, most experts agree that progenitor liver cells are activated, in response to significant exposure to hepatotoxins. Also, progenitor cells derived from nonhepatic sources, such as bone marrow and pancreas, have been demonstrated recently to be capable of differentiating into mature hepatocytes under correct microenvironmental conditions” (Gandillet et al., 2003). At present, analyses of human HCCs for oval cell markers, comparison of their gene-expression patterns with rat fetal hepatoblasts and the cellular characteristics of HCC from various animal models have provided contrasting results about the cellular origin of HCC and imply dual origins from either oval cells or mature hepatocytes. The failure to identify a clear cell of origin for HCC might stem from the fact that there are multiple cells of origin, perhaps reflecting the developmental plasticity of the hepatocyte lineage. The resolution of the HCC cell of origin issue could affect the development of useful preventative strategies to target nascent neoplasms, foster an understanding of how HCC-relevant genetic lesions function in that specific cell-development context and increase our ability to develop more accurate mouse models in which key genetic events are targeted to the appropriate cellular compartment (Farazi and DePinho, 2006). Two reviews by Librecht (2006) and Wu and Chen (2006) provide excellent summaries of the issues involved in identifying the target cell for HCC and the review by Roskams et al. (2004) provides a current view of the "oval cell" its location and human equivalent. Recent reports by Best and Coleman (2007) suggest another type of liver cell is also capable of proliferation and differentiating into small hepatocytes (i.e., small hepatocyte-like progenitor cell).

The review by Librecht (2006) provides an excellent description of the controversy and data supporting different views of the cells of origin for HCC.

> In recent years, the results of several studies suggest that human liver tumors can be derived from hepatic progenitor cells rather than from mature cell types. The available data indeed strongly suggest that most combined hepatocellular-cholangiocarcinomas arise from hepatic progenitor cells (HPCs) that retained their potential to differentiate into the hepatocyte and biliary lineages. Hepatic progenitor cells could also be the basis for some hepatocellular carcinomas and hepatocellular adenomas, although it is very difficult to determine the origin of an individual hepatocellular carcinoma. There is currently not enough data to make statements regarding a hepatic progenitor cell origin of cholangiocarcinoma. The presence of hepatic progenitor cell markers and the presence and extent of the cholangiocellular component are factors that are related the prognosis of hepatocellular carcinomas and combined hepatocellular-cholangiocarcinomas, respectively…The traditional view that adult human liver tumors arise from mature cell types has been challenged in recent decades…HPCs are small epithelial cells with an oval nucleus, scant cytoplasm and location in the bile ductules and canals of Hering. HPCs can differentiate towards the biliary and hepatocytic lineages. Differentiation towards the biliary lineage occurs via formation of reactive bile ductules, which are anastamosing ductules lined by immature biliary cells with a relatively large and oval nucleus surrounded by a small rim of cytoplasm. Hepatocyte differentiation leads to the formation of intermediate hepatocyte-like cells, which are defined as polygonal cells with a size intermediate between than of HPCs and hepatocytes. In most liver diseases, hepatic progenitor cells are "activated" which means that they proliferate and

differentiate towards the hepatocytic and/or biliary lineages. The extent of activation is correlated with disease severity…HPCs and their immediate biliary and hepatocytic progeny not only have a distinct morphology, but they also express several markers, with many also present in bile duct epithelial cells. Immunohistochemistry using antibodies against these markers facilitates the detection of HPCs. The most commonly used markers are cytokeratin (CK) 19 and CK7…The proposal that a human hepatocellular carcinoma does not necessarily arise from mature hepatocyte, but could have HPC origin, has classically been based on three different observations. Each of them, however, gives only indirect evidence that can be disputed…Firstly, it has been shown that HPCs are the cells of origin of HCC in some animal models of hepatocarcinogenesis, which has led to the suggestion that this might also be the case in humans. However, in other animal models, the HCCs arise from mature hepatocytes and not from HPCs or reactive bile ductular cells (Bralet et al 2002; Lin et al 1995– DEN treated rats). Since it is currently insufficiently clear which of these animal models accurately mimics human hepatocarcinogenesis, one should be careful about extrapolating data regarding HPC origin of HCC in animal models to the human situation…Secondly, liver diseases that are characterized by the presence of carcinogens and development of dysplastic lesions also show HPC activation. Therefore, the suggestion has been made that HPCs form a "target population" for carcinogens, but this is only a theoretical possibility not supported by experimental data…Thirdly, several studies have shown that a considerable proportion of HCCs express one or more HPC markers that are not present in normal mature hepatocytes. Due to the fact that most HPC markers are also expressed in the biliary lineage, the term "biliary marker" has been used in some of these studies. The "maturation arrest" hypothesis states that genetic alterations occurring in a HPC, or its immediate progeny, cause aberrant proliferation and prevent its normal differentiation. Further accumulation of genetic alterations eventually leads to malignant transformation of these incompletely differentiated cells. The resulting HCC expresses HPC markers as evidence of its origin. However, expression of HPC markers can also be interpreted in the setting of the "dedifferentiation" hypothesis, which suggests that the expression of HPC markers is acquired during tumor progression as a consequence of accumulating mutations. For example, experiments in which human HCC cells lines were transplanted into nude mice have nicely shown that the expression of HPC marker, CK19, steadily increased when the tumors became increasingly aggressive and metastasized to the lung, Thus, the expression of CK19 in a HCC does not necessarily mean that the tumor has a HPC origin, but it can also be mutation-induced, acquired expression associated with tumor progression. Both possibilities are not mutually exclusive. For an individual HCC that expresses a HPC marker, it remains impossible to determine whether this marker reflects the cellular origin and/or is caused by tumor progression. This can only be elucidated by determining whether HCC contains cells that are ultrastructurally identical to HPCs in nontumor liver.

Similarly, the review by Wu and Chen (2006) also presents a valuable analysis of these issues and state:

> The question of whether hepatocellular carcinomas arises from the differentiation block of stem cells or dedifferentiation of mature cells remains controversial. Cellular events during hepatocarcinogenesis illustrate that HCC may arise for cells at various stages of differentiation in the hepatic stem cell lineage…The role of cancer stem cells has been demonstrated for some cancers, such as cancer of the hematopoietic system, breast and brain. The clear similarities between normal stem cell and cancer stem cell genetic programs are the basis of the a proposal that some cancer stem cells could derived form human adult stem cells. Adult mesenchymal stem cells (MSC) may be targets for malignant transformation and undergo spontaneous transformation following long-term *in vitro* culture, supporting the hypothesis of cancer stem cell origin. Stem cells are not only units of biological organization, responsible for the development and the regeneration of tissue and organ systems, but are also targets of carcinogenesis. However, the origin of the cancer stem cell remains elusive…Three levels of cells that can respond to liver tissue renewal or damage have been proved (1) mature liver cells, as "unipotential stem cells," which proliferate under normal liver tissue renewal and respond rapidly to liver injury, (2) oval cells, as bipotential stem cells, which are activated to proliferate when the liver damage is extensive and chronic or if proliferation of hepatocytes is inhibited; and (3) bone marrow stem cells, as multipotent liver stem cells, which have a very long proliferation potential. There are two major nonexclusive hypotheses of the cellular origin of cancer; from stem cells due to maturation arrest or from dedifferentiation of mature cells. Research on hepatic stem cells in hepatocarcinogenesis has entered a new era of controversy, excitement and great expectations…The two major hypotheses about the cellular origination of HCC have been discussed for almost 20 years. Debate has centered on whether or not HCC originates from the differentiation block of stem cells or dedifferentiation of mature cells. Recent research suggests that HCC may originate from the transdifferentiation of bone marrow cells. In fact, there might be more than one type of carcinogen target cell. The argument about the origination of HCC becomes much clearer when viewed from this viewpoint: poorly differentiated HCC originate from bone marrow stem cells and oval cells, while well-differentiated HCC originates form mature hepatocytes…The cellular events during hepatocarcinogenesis illustrate that HCC may arise from cells at various stages of differentiation in the hepatocyte lineage. There are four levels of cells in the hepatic stem cell lineage: bone marrow cell, hepato-pancreas stem cell, oval cell and hepatocyte. HSC and the liver are known to have a close relationship in early development. Bone marrow stem cells could differentiate into oval cells, which could differentiate into heptatocytes and duct cells. The development of pancreatic and liver buds in embryogenesis suggests the existence of a common progenitor cells to both the pancreas and liver. All of the four levels of cells in the stem cell lineage may be targets of hepatocarcinogenesis.

Along with the cell types described as possible targets and participants in HCC, Best and Coleman (2007) describe yet another type of cell in the liver that can respond to hepatocellular injury, which they term small hepatocyte-like progenitor cells and conclude that they are not the progeny of oval cells, but represent a distinct liver progenitor cell population. Another potential regenerative cell is the small hepatocyte-like progenitor cell (SHPC). SHPCs share some phenotypes with hepatocytes, fetal hepatoblasts, and oval cells, but are phenotypically distinct. They express markers such as albumin, transferring, and alpha-fetoprotein (AFP) and possess bile canaliculi and store glycogen.

A recent review by Roskams et al. (2004) provides a current view of the "oval cell" its location and human equivalent. They conclude that

> while similarities exist between the progenitor cell compartment of human and rodent livers, the different rodent models are not entirely comparable with the human situation, and use of the same term has created confusion as to what characteristics may be expected in the human ductular reaction. For example, a defining feature of oval cells in many rodent models of injury is production of alpha-fetoprotein, whereas ductular reactions in humans rarely display such expression. Therefore we suggest that the "oval cell" and "oval –like cell" no longer be used in description of human liver.

In the chronic hepatitis and cancer model of Vig et al. (2006) it is not the oval cells or SHPCs that are proliferating but the mature hepatocytes, thus, supporting theories that it is not only oval cells that are causing proliferations leading to cancer. Vig et al. (2006) also report that studies in mice an humans indicate that oval cells also may give rise to liver tumors and that oval cells commonly surround and penetrate human liver tumors, including those caused by hepatitis B. Tarsetti et al. (1993) suggest that although some studies have suggested that oval cells are directly involved in the formation of HCC others assert that HCC originates from preneoplastic foci and nodules derived from hepatocytes and report that HCC evolved in their model of liver damage from hepatocytes, presumably hepatocellular nodules, and not from oval cells. They also suggest that proliferation alone may not lead to cancer. Recent studies that follow the progression of hepatocellular nodules to HCC in humans (see Section E.3.2.4, below) suggest an evolution from nodule to tumor.

E.3.1.5. *Status of Mechanism of Action for Human Hepatocellular Carcinoma (HCC)*

The underlying molecular mechanisms leading to hepatocarcinogenesis remain largely unclear (Yeh et al., 2007). Although HCC is multistep, and its appearance in children suggest a genetic predisposition exists, the inability to identify most of the predisposing genes and how their altered expression relates to histological lesions that are the direct precursors to HCC, has made it difficult to identify the rate limiting steps in hepatocarcinogenesis (Feitelson et al., 2002). Calvisi et al. (2007) report that although the major etiological agents have been identified, the molecular pathogenesis of HCC remains unclear and that while deregulation of a number of oncogenes (e.g., *c-Myc*, *cyclin D1* and *β-catenin* and tumor suppressor genes including *P16*^{INK4A}, *P53*, *E-cadherin*, *DLC-1*, and *pRb*) have been observed at different frequencies in HCC, the specific genes and the molecular pathways that play pivotal roles in liver tumor development have not been identified. Indeed rather than simple patterns of mutations, pathways that are common to cancer have been identified through study of tumors and through transgenic mouse models. Branda and Wands (2006) state that the molecular factors and interactions involved in hepatocarcinogenesis are still poorly understood but are particularly true with respect to genomic mutations, "as it has been difficult to identify common genetic changes in more than 20% to 30% of tumors." As well as phenotypically heterogeneous, "it is becoming clear that HCCs are genetically heterogeneous tumors." The descriptions of heterogeneity of tumors and of pathway disruptions common to cancer are also shown for liver tumors (see Sections E.3.1.6 and E.3.1.8, below). However, many of these studies focus on the end process and of examination of the genomic phenotype of the tumor for inferences regarding clinical course, aggressiveness of tumor, and consistency with other forms of cancer. As stated above, the events that produce these tumors from patients with conditions that put them at risk, are not known.

El-Serag and Rudolph (2007) suggest that risk of HCC increases at the cirrhosis stage when liver cell proliferation is decreased and that acceleration of carcinogenesis at this stage may result from telomere shortening (resulting in limitations of regenerative reserve and induction of chromosomal instability), impaired hepatocyte proliferation (resulting in cancer induction by loss of replicative competition), and altered milieu conditions that promote tumor cell proliferation.

> When telomeres reach a critically short length, chromosome uncapping induces DNA damage signals, cell-cycle arrest, senescence, or apoptosis. Telomeres are critically short in human HCC and on the single cell level telomere shortening correlated with increasing aneuploidy in human HCC…Chemicals inhibiting hepatocyte proliferation accelerate carcinogen-induced liver tumor formation in rats as well as the expansion and transformation of transplanted hepatocytes. It is conceivable that abnormally proliferating hepatocytes would not expand in healthy regenerating liver but would expand quickly and eventually transform in the growth restrained cirrhotic liver….Liver mass is controlled by growth factors – mass loss through could provide a growth stimulatory macroenvironment. For the microenvironment, cirrhosis activates stellate cells resulting in increased

production of extracellular matrix proteins, cytokines, growth factors, and products of oxidative stress.

Like other cancers, genomic instability is a common feature of human HCC with various mechanisms thought to contribute, including telomere erosion, chromosome segregation defects, and alteration in DNA damage-response pathways. In addition to genetic events associated with the development of HCC (p53 inactivation, mutation in β-catenin, overexpression of ErbB receptor family members, and overexpression of the MET receptor whose ligand is HGF) various cancer-relevant genes seem to be targeted on the epigenetic level (methylation) in human HCC (Farazi and DePinho, 2006). Changes in methylation have been detected in the earliest stages of hepatocarcinogenesis and to a greater extent in tumor progression (Lee et al., 2003). Seitz and Stickel (2006) report that aberrant DNA hypermethylation (a silencing effect on genes) may be associated with genetic instability as determined by the loss of heterozygosity and microsatellite instability in human HCC due to chronic viral hepatitis and that modifications of the degree of hepatic DNA methylation have also been observed in experimental models of chronic alcoholism. Farazi and DePinho (2006) report that two of the key molecules that involved in DNA damage response, p53 and BRCA2, seem to have roles in destabilizing the HCC genome (Collin, 2005). The inactivation of p53 through mutation or viral oncoprotein sequestration is a common event in HCC and p53 knock in mouse models containing dominant point mutations have been shown to cause genomic instability. However, Farazi and DePinho (2006) note that despite documentation of deletions or mutations in these and other DNA damage network genes, their direct roles in the genomic instability of HCC have yet to be established in many genetic model systems.

Telomere shortening has been described as a key feature of chronic hyperproliferative liver disease (Urabe et al., 1996; Miura et al., 1997; Rudolf and DePinho, 2001: Kitada et al., 1995), specifically occurring in the hepatocyte compartment. These observations have fueled speculation that telomere shortening associated with chronic liver disease and hepatocyte turnover contribute to the induction of genomic instability that drives human HCC (Farazi and DePinho, 2006). Defects in chromosome segregation during mitosis result in aneuploidy, a common cytogenetic feature of cancer cell including HCC (Farazi and DePinho, 2006).

Several studies have attempted to categorize genomic changes in relation to tumor state. In general, high levels of chromosomal instability seem to correlate with the de-differentiation and progression of HCC (Wilkens et al., 2004). Several studies have suggested certain chromosomal changes to be specific to dysplastic lesions, early –stage and late-stage HCCs, and metastases. It is important to note that the studies that have attempted to compare genomic profiles and tumor state are few in number, often did not classify HCCs on the basis of etiology, and used relatively low-resolution genome-scanning platforms (Farazi and DePinho, 2006). Farazi and DePinho (2006) note that it should be emphasized that although genome–etiology correlates reported in some studies, are intriguing, several studies have failed to uncover significant differences in genomic changes between different etiological groups, although the outcome might related to small sample sizes and the low-resolution genome–scanning platform used.

E.3.1.6. *Pathway and Genetic Disruption Associated with Hepatocellular Carcinoma (HCC) and Relationship to Other Forms of Neoplasia*

In their landmark paper, Hanahan and Weinberg (2000) suggested that the vast catalog of cancer cell genotypes were a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth; self-sufficiency in growth signals, insensitivity to growth –inhibitory (antigrowth signals), elevation of programmed cell death (apoptosis), limitless replication potential, sustained angiogenesis, and tissue invasion and metastasis. They proposed that these six capabilities are shared in common by most and perhaps all types of human tumors and, while virtually all cancers must acquire the same six hallmark capabilities, their means of doing so would vary significantly, both mechanistically and chronologically. It was predicted that in some tumors, a particular genetic lesions may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis. Loss of the p53 tumor suppressor was cited as an example that could facilitate both angiogenesis and resistance to apoptosis and to enable the characteristic of genomic instability. The paths that cells could take on their way to becoming malignant were predicted to be highly variable, and within a give cancer type, mutation of a particular target genes such as *ras* or *p53* could be found only in a subset of otherwise histologically identical tumors. Furthermore, mutations in certain oncogenes and tumor suppressor genes could occur early in some tumor progression pathways and late in others. Genes known to be functionally altered in "cancer" were identified as including Fas,Bcl2, Decoy R, Bax, Smads, TFGβR, p15, p16, Cycl D, Rb, human papilloma virus E7, ARF, PTEN, Myc, Fos, Jun, Ras, Abl, NF1, RTK, transforming growth factor alpha (TGF-α), Integrins, E-cadherin, Src, β-catenin, APC, and WNT.

Branda and Wands (2006) report that two signal transduction cascades that appear to be very important are insulin/IFG-1/IRS-1/MAPK and Wnt/Frizzled/β-catenin pathways which are activated in over 90% of HCC tumors (Branda and Wands, 2006). Feitelson et al. (2002) reported that

> In addition to NF-κB, up-regulated expression of rhoB has been reported in some HCCs. RhoB is in the *ras* gene family, is associated with cell transformation, and

may be a common denominator to both viral and non-viral hepatocarcinogenesis. Activation of ras and NF-κB, combined with down regulation of multiple negative growth regulatory pathways, then, may contribute importantly to early steps in hepatocarcinogenesis. Thus viral proteins may alter the patterns of hepatocellular gene expression by transcriptional trans-regulation…Another early event appears to involve the mutation of β-catenin, which is a component of the Wnt signal transduction pathway whose target genes include c-myc, c-jun, cyclin D1, fibronectin, the connective tissue growth factor WISP, and matrix metaolloproteinases.

Boyault et al. (2007) report that

> altogether, the principle carcinogenic pathways known to be deregulated in HCC are inactivation of TP53, Wnt/wingless activation mainly through CTNNB1 mutations activating β-catenin- and AXIN1-inactivating mutations, retinoblastoma inactivation through RB1 and CDKN2A promoter methylation and rare gene mutations, insulin growth factor activation through IGF2 overexpression, and IGF2R-inactiving mutations.

El-Serag and Rudolph suggest that "in general, the activation of oncogenic pathways in human HCC appears to be more heterogeneous compared with other cancer types." El-Serag and Rudolph (2007) report that the p53 pathway is a major tumor-suppressor pathway that (1) limits cell survival and proliferation (replicative senescence) in response to telomere shortening (2) induces cell-cycle arrest in response to oncogene activation (oncogene-induced senescence), (3) protects genome integrity, and (4) is affected at multiple levels in human HCC. "p53 mutations occur in aflatoxin induced HCC (>50%) and with lower frequency (20-40%) in HCC not associated with aflatoxin." In addition,

> the vast majority of human HCC overexpresses gankyrin, which inhibits both Rb checkpoint and p53 checkpoint function…The p16/Rb checkpoint is another major pathway limiting cell proliferation in response to telomere shortening, DNA damage, and oncogene activation. In human HCC the Rb pathway is disrupted in more than 80% of cases, with repression of p16 by promoter methylation being the most frequent alteration. Moreover, expression of gankyrin (an inhibitor of p53 and Rb checkpoint function) is increased in the vast majority of human HCCs, indicating that the Rb checkpoint is dysfunctional in the vast majority of human HCCs…The frequent inactivation of p53 in human HCC indicates that abrogation of p53-dependent apoptosis could promote hepatocarcinogenesis. The role of impairment of p53-independent apoptosis for hepatocarcinogenesis remains to be defined…Activation of the β-catenin pathway frequently occurs in mouse and human HCC involving somatic mutations, as well as transcriptional repression of negative regulators. An activation of the Akt

> signaling and impaired expression of phosphatase and tensin homolog (PTEN) (a negative regulator of Akt) have been reported in 40-60% of Human HCC.

They suggest that although *Myc* is a potent oncongene inducing hepatocarcinogenesis in mouse models the data on human HCC are heterogeneous and further studies are required.

E.3.1.7. *Epigenetic Alterations in Hepatocellular Carcinoma (HCC)*

The molecular pathogenesis of HCC remains largely unknown but it is presumed that the development and progression of HCC are the consequence of cumulative genetic and epigenetic events similar to those described in other solid tumors (Calvisi et al., 2006). Calvisi et al. (2007) provide a good summary of DNA methylation status and cancer as well as its status in regard to HCC:

> Aberrant DNA methylation occurs commonly in human cancers in the forms of genome-wide hypomethylation and regional hypermethylation. Global DNA hypomethylation (also known as demethylation) is associated with activation of protooncogenes, such as c-Jun, c-Myc, and c-HA-Ras, and generation of genomic instability. Hypermethylation on CpG islands located in the promoter regions of tumor suppressor genes results in transcriptional silencing and genomic instability. CpG hypermethylation (also known as de novo methylation) acts as an alternative and/or complementary mechanisms to gene mutations causing gene inactivation, and it is now recognized as an important mechanism in carcinogenesis. Although the mechanism(s) responsible for de novo methylation in cancer are poorly understood, it has been hypothesized that epigenetic silencing depends on activation of a number of proteins known as DNA methyltransferases (DNMTs) that posses de novo methylation activity. The importance of DNMTs in CpG methylation was substantiated by the observation that genetic disruption of both DNMT1 and DNMT3b genes in HCT116 cell lines nearly eliminated methyltransferase activity. However, more recent findings indicate that the HCT116 cells retain a truncated, biologically active form of DNMT1 and maintain 80% of their genomic methylation. Further reduction of DNMT1 levels by a siRNA approach resulted in decreased cell viability, increased apoptosis, enhanced genomic instability, checkpoint defects, and abrogation of replicative capacity. These data show that DNTM1 is required for cell survival and suggest that DNTM1 has additional functions that are independent of its methyltransferase activity. Concomitant overexpression of DNMT1, -3A, and -3b has been found in various tumors including HCC. However, no changes in the expression of DNMTs were found in other neoplasms, such as colorectal cancer, suggesting the existence of alternative mechanisms. In HCC, a novel DNMT3b splice variant, known as DNMT3b4 is overexpressed. DNMT3b4 lacks DNMT activity and competes with DNMT2b3 for targeting of pericentromeric satellite regions in HCC, resulting in DNA hypomethylation of these regions and induction of

chromosomal instability, further linking aberrant methylation and generation of genomic alterations.

It is now well accepted that methylation changes occur early and ubiquitously in cancer development. The case has been made that tumor cell heterogeneity is due, in part, to epigenetic variation in progenitor cells and that epigenetic plasticity together with genetic lesions drive tumor progression (Feinberg et al., 2006).

A growing number of genes undergoing aberrant CpG island hypermethylation in HCC have been discovered, suggesting that de novo methylation is an important mechanism underlying malignant transformation in the liver. However, most of the previous studies have focused on a single or a limited number of genes, and few have attempted to analyze the methylation status of multiple genes in HCC and associated chronic liver diseases. In addition, the functional consequence(s) of global DNA hypomethylation and CpG island hypermethylation in human liver cancer has not been investigated to date. Furthermore, to our knowledge no comprehensive analysis of CpG island hypermethylation involving activation of signaling pathways has been performed.

Calvisi et al. (2007) report that global gene expression profiles show human HCC to harbor common molecular features that differ greatly from those of nontumorous surrounding tissues, and that human HCC can be subdivided into 2 broad but distinct subclasses that are associated with length of patient survival. They further suggest that aberrant methylation is a major event in both early and late stages of liver malignant transformation and might constitute a critical target for cancer risk assessment, treatment, and chemoprevention of HCC. Calvisi et al. (2007) conducted analysis of methylation status of genes selected based on their capacity to modulate signaling pathways (*Ras*, Jak/Stat, Wingless/Wnt, and RELN) and/or biologic features of the tumors (proliferation, apoptosis, angiogenesis, invasion, DNA repair, immune response, and detoxification). Normal livers were reported to show the absence of promoter methylation for all genes examined. At least 1 of the genes involved in inhibition of Ras (*ARH1, CLU, DAB2, hDAB21P, HIN-1, HRASL, LOX, NORE1A, PAR4, RASSF1A, RASSF2, RASSF3, RASSF4, RIG, RRP22,* and *SPRY2* and *-4*), Jak/Stat (*ARH1,CIS, SHP1, PIAS-1, PIAS-γ, SOCS1, -2,* and *-3, SYK,* and *GRIM-19*), and Wnt/β-catenin (*APC, E-cadherin, γ-catenin, SFRP1, -2, -4,* and *-5, DKK-1* and *-3, WIF-1* and *HDPR1*) pathways was affected by de novo methylation in all HCC. A number of these genes were also reported to be highly methylated in the surrounding nontumorous liver. In contrast, inactivation of at least 1 of these genes implicated in the RELN pathway (DAB1, reelin) was detected differentially in HCC of subclasses of tumor that had difference in tumor aggressiveness and progression. Epigenetic silencing of multiple tumor suppressor genes maintains activation of the *Ras* pathway with a major finding in the Calvisi et al. (2007) study to be the concurrent hypermethylation of multiple inhibitors of the *Ras* pathway with *Ras* was significantly more active in HCC than in surrounding or normal livers. Also important, was the finding that no significant associations between methylation patterns and specific etiologic agents (i.e., HVB, HVC, ethanol, etc.) were detected further substantiating the conclusion that aberrant methylation is a ubiquitous phenomenon in hepatocarcinogenesis.

> Current evidence suggests that hypomethylation might promote malignant transformation via multiple mechanisms, including chromosome instability, activation of protooncogenes, reactivation of transposable elements, and loss of imprinting…The degree of DNA hypomethylation progressively increased from nonneoplastic livers to fully malignant HCC, indicating that genomic hypomethylation is an important prognostic factor in HCC, as reported for brain, breast, and ovarian cancer.

Calvisi et al. (2007) also report that regional CpG hypermethylation was also enhanced during the course of HCC disease and that the study of tumor suppressor gene promoters showed that CpG methylation was frequently detected both in surrounding nontumorous livers and HCC.

E.3.1.8. *Heterogeneity of Preneoplastic and Hepatocellular Carcinoma (HCC) Phenotypes*

A very important issue for the treatment of HCC in humans is early detection. Research has focused on identification of lesions that will progress to HCC and to also determine from the phenotype of the nodule and genetic expression its cell source, likely survival, and associations with etiologies and MOAs. As with rodent models where preneoplastic foci have been observed to be associated with progression to adenoma and carcinoma, nodules observed in humans with high risk for HCC have been observed to progress to HCC. In humans, histomorphology of HCC is notoriously heterogeneous (Yeh et al., 2007). Although much progress has been made, there is currently not universally accepted staging system for HCC partly because of the natural course of early HCC is unknown and the natural progression of intermediated and advanced HCC are quite heterogeneous (Thorgeirsson, 2006). Nodules are heterogeneous as well with differences in potential to progress to HCC. Chen et al. (2002) report that standard clinical pathological classification of HCC has limited valued in predicting the outcome of treatment as the phenotypic diversity of cancer is accompanied by a corresponding diversity in gene expression patterns. There is also histopathological variability in the presentation of HCC in geographically diverse regions of the world with some slow growing, differentiated HCC nodules surrounded by a fibrous capsule are common among Japanese but, in contrast, a "febrile" form of HCC, characterized by leukocytosis, fever, and necrosis within a poorly differentiated tumor to be common in South African blacks (Feitelson et al., 2002).

A multistep process is suggested histologically, where HCC appears within the context of chronic hepatitis and/or cirrhosis within regions of the liver cell dysplasia or adenomatous hyperplasia (Feitelson et al., 2002). Kobayashi et al. (2006) report that the higher the grade of the nodule the higher the percentage that will progress to HCC with 18.8% of all nodules and regenerative lesions going on to become HCC, 53.3% remaining unchanged, and 27.9% disappearing in the observation period of 0.1 to 8.9 years. Borzio et al. (2003) report that the rate of liver malignant transformation was 40% in larger regenerative nodules, low-grade dysplastic, and high-grade dysplastic nodules with higher grade of dysplasia extranodular detection of large cell change and hyperchronic pattern associated with progression to HCC. Yeh et al. (2007) report that nuclear staining for Ki-67 and Topo II-α (a nuclear protein targeted by several chemotherapeutic agents) significantly increased in the progression from cirrhosis, through high grade dysplastic nodules to HCC whereas the scores for TGF-α in these lesions showed an inverse relationship. "In comparison with 18 HCC arising in noncirrhotic livers, the expression of TGF-α is significantly stronger in cirrhotic liver than in noncirrhotic parenchyma and its expression is also stronger in HCC arising in cirrhosis than in HCC arising in noncirrhotic patients." They concluded that initiation in cirrhotic and noncirrhotic liver may have different pathways with Transforming growth factor-α (a mitogen activated the EFGR) playing a relative more important role in HCC from cirrhotic liver. Over expression of TGF-α in the liver of transgenic mice induced increased proliferation, dysplasia, adenoma and carcinoma. Yeh et al. (2007) concluded that such high-grade dysplastic nodules are precursor lesions in hepatocarcinogenesis and that TGF-α may play an important role in the early events of liver carcinogenesis.

Moinzadeh et al. (2005) reported in a meta-analysis of all available (n = 785) HCCs that gains and losses of chromosomal material were most prevalent in a number of chromosomes and that amplifications and deletions occurred on chromosomal arms in which oncogenes (e.g., MYC and 8q24) and tumor suppressor genes (e.g., RB1 on 13q14) are located as well a modulators of the WNT-signaling pathway. However, in multifocal HCC, nodules arising de novo within a single liver have a different spectrum of genetic lesions. "Hence, there are likely to be many paths to hepatocellular carcinoma, and this is why it has been difficult to assign specific molecular alterations to changes in hepatocellular phenotype, clinical, or histopathological changes that accompany tumor development" (Feitelson et al., 2002).

Serum AFP is commonly used as tumor marker for HCC. Several reports have linked HCC to cytokines in an attempt to find more specific markers of HCC. Jia et al. (2007) report that AFP marker allows for identification of a small set of HCC patients with smaller tumors, and these patients have a relatively long-term survival rate following curative treatment.

> Presently the only approach to screen for the presence of HCC in high-risk populations is the combination of serum AFP and ultrasonagraphy. However, elevated AFP is only observed in about 60 to 70% of HCC patients and to a lesser extent (33-65%) in patients with smaller HCCs. Moreover, nonspecific elevation of serum AFP has been found in 15% to 58% of patients with chronic hepatitis and 11% to 47% of patients with liver cirrhosis.

Soresi et al. (2006) report that serum interleukin (IL)-6 levels are low in physiological conditions, but increase considerably pathological conditions such as trauma, inflammation and neoplasia. In tumors IL-6 may be involved in promoting the differentiation and growth of target cells. "Many works have reported high serum IL-6 levels in various lifer diseases such as acute hepatitis, primary biliary cirrhosis, chronic hepatitis (hepatitis C) and HCV-correlated liver cirrhosis and in hepatocellular carcinoma." Soresi et al. (2006) report that patients with HCC group had higher IL-6 values than those with cirrhosis and that "higher-staged" patients had the highest IL-6 levels. Hsia et al (2007) also examined IL-6, IL-10 and hepatocyte growth factor (HGF) as potential markers for HCC.

> The expression of IL-6 or IL-10 or higher level of HGF or AFP was observed only 0-3% of normal subjects. Patients with HCC more frequently had higher IL-6 and IL-10 levels, where as HGF levels in HCC patients were not significantly elevated compared to patients with chronic hepatitis or non-HCC tumors (but greater than controls). Among patients with low AFP level, IL-6 or IL-10 expression was significantly associated with the existence of HCC. Patients with large HCC (>5 cm) more often had increased IL-6, IL-10 or AFP levels. Serum levels of IL-6 and IL-10 are frequently elevated in patients with HCC but not in benign liver disease or non-HCC tumors.

Nuclear DNA content and ploidy have also been the subjects of several studies through the years for identification of pathways for prediction of survival or origin of tumors. Nakajima et al. (2004) report that p53 loss can contribute to the propagation of damaged DNA in daughter cells through the inability to prevent the transmission of inaccurate genetic material, considered to be one of the major mechanisms for the emergence of aneuploidy in tumors with inactivated p53 protein and the increasing ploidy in HCC was associated with disturbance in p53. McEntee et al. (1991) reported that specimens from 74 patients who underwent curative resection for primary HCC and analyzed for DNA content, (i.e., tumors were classified as DNA aneuploid if a separate peak was present from its standard large diploid peak [2C] and tetraploid peak [4C]) 33% were DNA diploid, 30% were DNA tetraploid/polyploidy, and 37% were aneuploid of the primary tumors examined. Nontumor controls were diploid and survival was not different between patients with diploid versus nondiploid tumors. Zeppa et al. (1998) reported ploidy in

84 hepatocellular carcinomas diagnosed by fine-needle aspiration biopsy to have 68 cases that were aneuploid and 16 euploid (9 diploid and 7 polyploid), with median survival of 38 months for patients with diploid HCC and 13 months for aneuploid HCC. Lin et al. (2003) report in their study of fine needle aspiration of HCC that

> the ratio of S and G2/M periods of DNA, which reflect cell hyperproliferation, in the group with HCC tumors> 3cm in diameter were markedly higher than those of the group with nodules< 3 cm in diameter and the group with hyperplastic nodules…DNA analysis of aspiration biopsy tissues acquired from intrahepatic benign hyperplastic nodules showed steady diploid (2c) peak that stayed in G1 period. DNA analysis of aspiration biopsy tissues acquired from HCC nodules showed S period of hyperproliferation and G2/M period. The DNA analysis of HCC nodules showed aneuploid peak.

They concluded that in regard to the biological behavior of the cell itself, that the normal tissue, reactive tissue and benign tumor all have normal diploid DNA but, like most other malignant tumors, "HCC appears to have polyploid DNA, especially aneuploid DNA." Attallah et al. (1999) report small needle liver biopsy data to show HCC to be 21.4% diploid, 50% aneuploid and 28.6% tetraploid and that higher ploidies (aneuploid and tetraploid) were observed in human liver cancer than residual tissues, although in some cases there was increased aneuploidy (cirrhosis, 37%, hepatitis ~50%). Of note for the study is the lack of appropriate control tissue and uncertainty as to how some of their diploid cells could have been binucleate tetraploid cells. Anti et al. (1994) reported reduction in binuclearity in the chronic hepatitis and cirrhosis groups that was significantly correlated with a rise in the diploid/polyploidy ratio and that precancerous and cancerous nodules within cirrhotic liver show an increased tendency toward diploidy or the emergence of aneuploid populations. They note that a number of investigators have noted significantly increased hepatocyte diploidization during the early stages of chemically induced carcinogenesis in rat liver, but other experimental findings indicate that malignant transformation can occur after any type of alteration in ploidy distribution. On the other hand, Melchiorri et al. (1994) note that several studies using flow cytometric or image cytometric methods reported high DNA ploidy values in 50−77% of the examined HCCs and that the presence of aneuploidy was significantly related to a poor patient prognosis. They report that the DNA content of mononucleated and binucleated hepatocytes, obtained by ultrasound-guided biopsies of 10 macroregenerative nodules without histologic signs of atypia from the lesions with the greater fraction of mononucleated hepatocytes were diagnosed as HCCs during the clinical follow-up with results also suggesting that diploid and tetraploid stem cell lines are the main lines of the HCCs as well as a reduction in the percentage of binucleated hepatocytes in HCC. Gramantieri et al. (1996) report that the percentage of binucleated cells was reduced in most of HCC they studied (i.e., the mean percentage of binucleated cells 9% in comparison to 24% found in normal liver) and that most HCC, as many other solid neoplasms, showed altered nuclear parameters.

Along with reporting pathways that are perturbed in HCC, emerging evidence also shows that signatures of pathway are predictive of clinical characteristics of HCC. A number of studies have examined gene expression in tumors to try to determine which pathways may have been disturbed in an attempt to predict survival and treatment options for the patients and to investigate possible MOAs for the tumor induction and progression. Chen et al. (2002) described a systematic characterization of gene expression patterns in human liver cancers using cDNA microarrays to study tumor and nontumor liver tissues in HCC patients, and of note did quality assurance on their microarray chips (many studies do not report that they have done so), and examined the effects of hepatitis virus on its subject and identified people with it. Most importantly, Chen et al. (2002) provided phenotypic anchoring of each tumor with its genetic profile rather than pooling data. The hierarchical analysis demonstrated that clinical samples could be divided into two major clusters, one representing HCC samples and the other with a few exceptions, representing nontumor liver tissues. Most importantly, expression patterns varied significantly among the HCC and nontumor liver samples and that samples from HBV-infected, hepatitis C virus infected, and noninfected individuals were interspersed in the HCC branch. Thus, tumors from people infected with HVB, HVC and noninfected people with HCC were interspersed in the HCC pattern and could be discerned based on etiology. One cluster of genes was highly expressed in HCC samples compared with nontumor liver tissues included a "proliferation cluster" comprised of genes whose functions are required for cell-cycle progression and whose expression levels correlate with cellular proliferation rates with most of the genes in this cluster are specifically expressed in the G2/M phase. Gene profiles for HCC were consistent with fewer molecular features of differentiated normal hepatocytes. Chen et al. (2002) noted that both normal and liver tumors are complex tissue compose of diverse cells and that distinct patterns of gene expression seemed to provide molecular signatures of several specific cell types including expression of two clusters of genes associated with T and B lymphocytes, presumably reflecting lymphocytic infiltration into liver tissues, and genes associated with stellate cell activation. This important finding acknowledges that HCC are not only heterogeneous in hepatocyte phenotype but are made up of many other nonparenchymal cell types and that gene expression patterns reflect that heterogeneity. A gene cluster was also identified at a higher level in HCC that included several genes typically expressed in endothelial cells, including CD34, which is expressed in endothelial cells in veins and arteries but not in the endothelial cells of the sinusoids in nontumor liver and which may reflect disruption of the molecular program that normally regulate blood vessel morphogenesis in the liver.

Of great importance was the investigation by Chen et al. (2002) of whether samples from multiple sites in a single HCC tumor, or multiple separate tumor nodules in one patient, would share a recognizable gene expression signature. With a few instructive exceptions, all the tumor samples from each patient clustered were reported to cluster together. To further examine the relationship among multiple tumor samples from individual patients, they calculated the pairwise comparison for all pairs of samples and samples some primary tumors multiple times. Tumor patterns of gene expression were more highly correlated those seen in samples from the same patient than other patients but every tumor had a distinctive and characteristic gene expression pattern, recognizable in all samples taken from different areas of the same tumor. For multiple discrete tumor masses obtained from six patients, three of these patients had multiple tumors with a shared distinctive gene expression pattern but in three other patients, expression patterns varied between tumor nodules and the difference provided new insights into the sources of variation in molecular and biological characteristics of cancers. Thus, in some patients multiple tumors were from the same clone, as demonstrated by a similar gene expression profile, but for some patients multiple tumors were arising from differing clones within the same liver. In regard to whether the distinctive expression patterns characteristic of each tumor reflect the individuality of the tumor or are determined by the patient in whom the tumor arose, analysis of the expression patterns observed in the two tumor nodules from one patient showed that the two tumors were not more similar than those of an arbitrary pair of tumors from different patients. These results show the heterogeneity of HCC and that “one gene pattern” will not be characteristic of the disease.

However, HCC did have a pattern that differed from other cancers. Chen et al. (2002) analyzed the expression patterns of 10 randomly selected HCC samples and 10 liver metastases of other cancers and reported that the HCC samples and the metastatic cancers clustered into two distinct groups, based on difference in their patterns of gene expression. Although some of the HCC samples were poorly differentiated and expressed the genes of the liver-specific cluster at very low levels compared to with either normal liver or well-differentiated HCC, the genes of the liver-specific cluster were reported to be consistently expressed at higher levels in HCC than in tumors of nonliver origin. Metastatic cancers originating from the same tissue typically clustered together, expressing genes characteristic of the cell types of origin. Thus, liver cancer was distinguishable from other cancer even though very variable in expression and differentiation state.

In an attempt to create molecular prognostic indices that can be used for identification of distinct subclasses of HCC that could predict outcome, Lee et al. (2004a) report two subclasses of HCC patients characterized by significant differences in the length of survival. They also identified expression profiles of a limited number of genes that accurately predicted the length of survival. Total RNAs from the 19 normal livers, including "normal liver in HCC patients," were pooled and used as a reference for all microarray experiments and thus variations between patients, and especially differences due to conditions predisposing HCC, were not determined. DNA microarray data using hierarchical clustering was reported to yield two major clusters, one representing HCC tumors, and the other representing nontumor tissues with a few exceptions that were not characterized by the authors. Lee et al. (2004a) report that, along with 2 distinctive subtypes of gene expression patterns in HCC, there was heterogeneity among HCC gene expression profiles and that one group had an overall survival time of 30.8 months and the other 83.7 months. Only about half the patients in each group were reported to have cirrhosis. Expression of typical cell proliferation markers such as PCNA and cell cycle regulators such as *CDK4, CCNB1, CCNA2,* and *CKS2* was greater in one class than the other of HCC.

The report by Boyault et al. (2007) attempted to compare etiology and genetic characterization of the tumors they produce and confirms the heterogeneity of HCC, some without attendant genomic instability. Boyault et al. (2007) reported that genetic alterations are indeed closely associated with clinical characteristics of HCC that define 2 mechanisms of hepatocarcinogenesis.

> The first type of HCC was associated with not only a high level of chromosome instability and frequent TP53 and AXIN1 mutations but also was closely linked to HBV infections and a poor prognosis. Conversely, the second subgroup of HCC tumors was chromosome-stable, having a high incidence of activating β-catenin alteration and was not associated with viral infection.

Boyault et al. (2007) reported that in a series of 123 tumors, mutations in the CTNNB1 (encoding β-catenin), TP53, ACIN1, TCF1, PIK3CA and KRAS genes in 34, 31, 13, 5, 2, and 1 tumors were identified, respectively. No mutations were found in NRAS, HRAS, and EGFR. Hypermethylation of the CDKN2A and CDH1 promoter was identified in 35 and 16% of the tumors, respectively. Boyault et al. (2007) grouped tumors by genomic expression as well as other factors. HCC groups associated with high rate of chromosomal instability were reported to be enriched with over expression of cell-cycle/proliferation/DNA metabolism genes. They concluded that "the primary clinical determinant of class membership is HBV infection and the other main determinants are genetic and epigenetic alterations, including chromosome instability, CTNNB1 and TP53 mutations, and parental imprinting. Tumors related to HCV and alcohol abuse were interspersed across subgroups G3-G6." Boyault et al. (2007) suggested that there results indicate that HBV infection early in life leads to a specific type of HCC that has immature features with abnormal parental gene imprinting selections, possibly through the persistence of fetal hepatocytes or alternatively through partial dedifferentiation of adult hepatocytes. "These G1 tumors are related to high-risk populations found in epidemiological studies."

E.3.2. Animal Models of Liver Cancer

There are obvious differences between rodents and primate and human liver, and there is a difference in background rates of susceptibility to hepatocarcinogenesis. With strains of mice there are large differences in responses to hepatotoxins (e.g., acetaminophen) and to hepatocarcinogens as well as background rates of hepatocarcinogenecity. Maronpot (2007) reports that modulators of murine hepatocarcinogenesis, such as diet, hormones, oncogenes, methylation, imprinting, and cell proliferation/apoptosis are among multiple mechanistically associated factors that impact this target organ response in control as well as in treated mice, and suggests that there is no one simple paradigm to explain the differential strain sensitivity to hepatocarcinogenesis. Because of the variety of studies with differing protocols used to generate susceptibility data, direct comparisons among strains and stocks is problematic but in regard to susceptibility to carcinogenicity the C3H/HeJ and C57BL/6J mouse have been reported to have up to a 40-fold difference in liver tumor multiplicity (Maronpot, 2007). However, as noted above, TCE causes liver tumors in C6C3F1 and Swiss mice with studies of trichloroethylene metabolites dichloroacetic acid, trichloroacetic acid, and CH suggesting that both dichloroacetic acid and trichloroacetic acid are involved in trichloroethylene-induced liver tumorigenesis. Many effects reported in mice after dichloroacetic acid exposure are consistent with conditions that increase the risk of liver cancer in humans and can involve GST Xi, histone methylation, and overexpression of insulin-like growth factor-II (IGF-II; Caldwell and Keshava, 2006). The heterogeneity of liver phenotype observed in mouse models is also consistent with human HCC. These data lend support to the qualitative relevance of the mouse model for TCE-induced cancer risk.

Bannasch et al. (2003) made important observations that have implications regarding the differences in susceptibility between rodent and human liver cancer. They stated that

> Although the classification of such nodular liver lesions in rodents as hyperplastic or neoplastic has remained controversial, persistent nodules of this type are considered neoplasms, designated as adenomas. In human pathology, the situation appears to be paradoxical because adenomas are only diagnosed in the

> noncirrhotic liver, yet a confusing variety terms avoiding the clearcut classification as an adenoma has been created for nodular lesions in liver cirrhoses, not withstanding that the vast majority hepatocellular carcinomas develop in cirrhotic livers. Even if a portion of these nodular lesions would be regarded as adenomas, being integrated into an adenoma-carcinoma sequence as observed in many animal experiments, clinical and epidemiological records of liver neoplasms, including both benign and malignant forms, would increase considerably. This would not only bring hepatic neoplasia further into focus of human neoplasia in general, but also shed new light on the classification of some chemicals producing high incidence of liver neoplasms in rodents, but appearing harmless to humans according to epidemiological evaluations solely based on the incidence of hepatocellular carcinoma in exposed populations.

Thus, that in humans only HCCs are recorded but in animals adenomas are counted as neoplasms, may indicate that the scope of the problem of liver cancer in humans may be underestimated.

Tumor phenotype differences have been reported for several decades through the work of Bannasch et al. The predominant cell line of foci of altered hepatocytes (FAH) have excess glycogen storage early in development that appears to be similar to that shown by DCA treatment. Bannasch et al. (2003) report that "the predominant glycogenotic-basophilic cell line FAH reveals that there is an overexpression of the insulin receptor, the IGF-1 receptor, the insulin receptor substrates-1/2 and other components of the insulin-stimulated signal transduction pathway." Bannasch states that foci of this type have increased expression of GST-π and insulin has also been shown to induce the expression of GST-pi but that hyperinsulin-induced foci do not show increased GST-π. Cellular dedifferentiation during progression from glycogenotic to basophilic cell populations is associated with downregulation in insulin signaling. The amphophilic-basophilic cell lineage of peroxisome proliferators and hepadnaviridae were reported to have foci that mimic effects of thyroid hormone with mitochondrial proliferation and activation of mitochondrial enzymes. Bannasch et al. (2003) state that

> the unequivocal separation of 2 types of compounds, usually classified as initiators and promoters, remains a problem at the level of the foci because at least the majority of chemical hepatocarcinogens seem to have both initiating and promoting activity, which may differ in quantitative rather than qualitative terms from one compound to another…Whereas genetic mutations have been predominantly postulated to initiate hepatocarcinogenesis for many years, more recently epigenetic changes have been increasingly discussed as a plausible cause of the evolution of preneoplastic foci characterized by metabolic changes including the expression of GSTpi.

Su and Bannasch (2003) report that glycogen-storing foci represents early lesion with the potential to progress to more advance glycogen-poor basophilic lesions through mixed cell foci and resulting hyperproliferative lesions and are associated with HCC in man. Small-cell change (SCC) of liver parenchyma (originally called liver cell dysplasia of small cell size) is reported to share cytological and histological similarities to early well defined HCC. Close association between SCC and more advanced (basophilic) foci indicates that foci often progress to HCC through SCC in humans. SCC were reported to be present in all basophilic foci. Previous studies were cited that showed that the biochemical phenotype of human FAH, mainly including glycogen storing clear cell foci and clear cell-predominated mixed cell foci, were observed in more than 50% of cirrhotic livers with or without HCC. FAH of clear and mixed cell types were observed in almost all livers bearing HCC, and in chronic liver diseases without HCC but at a lower frequency. Su and Bannasch (2003) report that

> the finding of mixed cell foci (MCF) mainly in livers with high-risk or cryptogenenic cirrhosis indicates that these are more advanced precursor lesions in man, in line with earlier observations in experimental animals. Considering their preferential emergence in cirrhotic livers of the high-risk group, their unequivocally elevated proliferative activity, and the resulting large size with frequent nodular transformation, we suggest that mixed cell populations are endowed with a high potential to progress to HCC in humans, as previously shown in rats.

In human HCC, irregular areas of liver parenchyma with marked cytoplasmic amphophilia, phenotypically similar to the amphophilic preneoplastic foci in rodent liver exposed to different hepatocarcinogenic chemicals (e.g., DHEA a peroxisome proliferator) or the hepadnaviruses were reported to present in 45% of the specimens from cirrhotic livers examined. "However, more data are needed to elucidate the nature of the oncocytic and amphophilic lesions regarding their role in HCC development."

With respect to the ability respond to a mitogenic stimulus, differences between primate and rodent liver response to a powerful stimulus, such as partial hepatectomy, have been noted that indicate that primate and human liver respond differently (and much more slowly) to such a stimulus. Gaglio et al. (2002) report after 60% partial hepatectomy in Rhesus macaques (*Macaca mulatto*), the surface area of the liver remnant was restored to its original preoperative value over a 30 day period. The maximal liver regeneration occurred between days 14 and 21, with thickening of liver cell plates, binucleation of hepatocytes, Ki-67 and PCNA expression (occurring in hepatocytes throughout the lobule at a maximum labeling index of 30%), and mitoses parallel increased most prominently between posthepatectomy days 14 and 30.

However, cytokines associated with inducing proliferation were elevated much earlier. TGF-α, IL-6, HGF, IL-6 and TNF-α mRNA persisted until Day 14, with peak elevations of IL-6, TNF-α, occurring 24 hours later surgery, and IL-6 reduced to control levels by Day 14. Gaglio et al. (2002) suggest that their results clearly indicate that the pattern and timing of liver regeneration observed in this nonhuman primate model are significantly different when comparing different species (e.g., peak expression of Ki-67 in a 60% partial hepatectomy model in rats occurs within hours following partial hepatectomy) and that the difference in timing and pattern of maximal hepatocellular regeneration cannot be explained simply by differences in size of animals (e.g., 60% partial hepatectomy in dogs produced liver regeneration peaks at 72 hours with weights approximating the weights of the Rhesus macaques). They note that previous studies in humans, who underwent 40−80% partial hepatectomy, reveal a similar delay in peak liver regeneration based on changes in serum levels of ornithine decarboxylase and thymidine kinase, further highlighting significant interspecies differences in liver regeneration. For C57BL/6 X 129 mice Fujita et al. (2001) report that after partial hepatectomy, the liver had recovered more than 90% of its weight within 1 week. This difference in response to a mitogenic stimulus has impacts on the interpretations of comparisons between rodent and primate liver responses to chemical exposures which give a transient increases in DNA synthesis or cell proliferation such as PPARα agonists. Also, as stated above, the primate and human liver, while having a significant polyploidy compartment, do not have the extent of polyploidization and the early onset of that has been observed in the rodent. However, as noted by Lapis et al. (1995), exposure to DEN has proven to be a highly potent hepatocarcinogen in nonhuman primates, inducing malignant tumors in 100% of animals with an average latent period of 16 months when administered at 40 mg/kg intraperitoneally every 2 weeks.

In regard to species extrapolation of epigenomic changes between humans and rodents, Weidman et al. (2007) caution that

> Although we do predict some overlap between mouse and human candidate imprinted genes identified through our machine-learning approach, it is likely that the most significant criterion in species-specific identification will differ. This difference underscored the importance for increased caution when assessing human risk from environmental agents that alter the epigenome using rodent models; the molecular pathways targeted may be independent.

Despite species differences, the genome of the mouse has been sequenced and many transgenic mouse models are being used to study the consequences of gene expression modulation and pathway perturbation to study human diseases and treatments. However, the use of transgenic models must be used with caution in trying to determine to determine MOAs and the background effects of the transgene (including background levels of toxicity) and specificity of effects must be taken into account for interpretation of MOA data, especially in cases where the knockout in the mouse causes significant liver necrosis or steatosis (Keshava and Caldwell, 2006; Keshava and Caldwell, 2006; Caldwell and Keshava, 2006; Caldwell et al., 2008b). For the determination of effects of pathway perturbation and similarity to human HCC phenotype, mouse transgenic models have been particularly useful with tumors produced in such models shown to correlate with tumor aggressiveness and survival to human counterparts.

E.3.2.1. *Similarities with Human and Animal Transgenic Models*

Mice transgenic for transforming growth factor –α (a member of the EGF family and a ligand for the ErfB receptors) develop HCCs (Farazi and DePinho, 2006). Compound TGFα and MYC transgenic mice show increase hepatocarcinogenesis that is associated with the disruption of TGF-β1 signaling and chromosomal losses, some of which are syntenic to those in human HCCs that include the retinoblastoma (RB) tumor suppressor locus (Sargent, 1999). Lee et al. (2004b) investigated whether comparison of global expression patterns of orthologous genes in human and mouse HCCs would identify similar and dissimilar tumor phenotypes, and thus, allow the identification of the best-fit mouse models for human HCC. The molecular classification of HCC on the basis of prognosis in Lee et al. (2004a) was further compared with gene-expression profiles of HCCs from seven different mouse models (Lee et al., 2004b). Lee et al. (2004b) characterized the gene expression patters of 68 HCC from seven different mouse models; two chemically induced (Ciprofibrate and diethylnitrosamine), four transgenic (targeted overexpression of *Myc, E2F1, Myc and E2F1*, and *Myc* and *Tgfa* in the liver). HCCs from some of these mice (MYC, E2F1 and MYC-E2F1 transgenics) showed similar gene-expression patterns to the ones of HCCs from patients with better survival. Murine HCCs derived for MYC-TGF-α transgenic model or diethylnitrosamine-treated mice showed similar gene-expression patterns to HCCs from patients with poor survival. The authors report that Myc Tgfa transgenic mice typically have a poor prognosis, including earlier and higher incident rates of HCC development, higher mortality, higher genomic instability and higher expression of poor prognostic markers (e.g., AFP) and that Myc and Myc/E2f1 transgenic mice have relatively higher frequency of mutation in β-catenin (*Catnb*) and nuclear accumulation of β-catenin that are indicative of lower genomic instability and better prognosis in human HCC.

Lee et al. (2004b) indentified three distinctive HCC clusters, indicating that gene expression pattern of mouse HCC are clearly heterogeneous and reported that Ciprofibrate-induced HCCs and HCCs from Acox -/- mice were closely clustered and well separated from other mouse models. However, are several issues regarding this study that give limitations to some of its conclusions regarding the Acox -/- mouse and Ciprofibrate treatment. The Acox -/- mouse is characterized by profound hepatonecrosis, which confounds conclusions regarding gene expression related to PPARα agonism made by the authors. There was very limited reporting of the animal models (DEN and Clofibrate) protocols used. Only three tumors were examined for Clofibrate treatment and it is unknown if the tumors were from the same animals. Similarly only three tumors were examined from DEN treatment, which has been shown to produce heterogeneous tumors and to produce necrosis in some paradigms of exposure. Myc/E2F1 and E2F1 mice were split in both clusters that were compared with human HCCs. The authors used previously published data from Meyer et al. (2003) for tumors from Acox1^{-1-} null mice, DENA-treated mice and Ciprofibrate-treated mice.

Meyer et al. (2003) examined three tumors from 2 C57BL/6j mice fed Ciprofibrate for 19 months and three tumors from 2 C57BL/6j mice injected with DEN at 2−3 months but the age at which tumors appear was not given by the authors. Pooled mRNA from animals of varying age (5−15 months old) was used for controls. mRNAs that differed by 2-fold in tumors were reported to be: 60 genes up-regulated and 105 genes down-regulated in Acox1^{-1-} null mice tumors; 136 genes up-regulated and 156 genes down-regulated in Ciprofibrate-induced tumors; and 61 genes up-regulated and 105 genes down-regulated in DEN-induced tumors. The authors state that "Each tumor class revealed a somewhat different unique expression pattern." There were "genes that were general liver tumor markers in all three types of tumors" with 38 genes commonly deregulated in all three tumor types. On note, the cell cycle genes (CDK4, CDC25Am CDC7 and MAPK3) cited by Lee et al. (2004b) as being more highly expressed in DEN-induced tumors were not reported to be changed in DEN tumors in Meyer et al. (2003) or to be altered in the Acox1^{-1-} null mice or mice treated with Ciprofibrate. Finally, the distinction between groups may be dominated by gene expression changes in a large number of genes that are related to PPAR activation but not related to hepatocarcinogenesis.

Calvisi et al. (2004a) used transgenic mice to study pathway alterations and tumor phenotype and to further examine the premise that genomic alterations (genetic and epigenetic) characteristic of HCC can describe tumors into 2 broad categories, the first category characterized by activation of the Wnt/Wingless pathway via disruption of β-catenin function and chromosomal stability and the second by chromosomal instability. Increased coexpression of c-myc with TGF-α or E2F-1 transgenic mice was reported to result in a dramatic synergistic effect on liver tumor development when compared with respective monotransgenic lines, including shorter latency period, and more aggressive phenotype whereas β-catenin activation is relatively common in HCCs developed in c-myc and c-myc/TGF-β1 transgenic mice, rare in the c-myc/TGF-α transgenic line which also has genomic instability. Calvisi et al. (2004a) also report that β-catenin staining correlated with histopathologic type of liver tumors. Eosinophilic tumors with abnormal nuclear staining of β-catenin were predominant in neoplastic lesions characteristic of c-myc and c-myc/E2F1 lesions. Poorly differentiated HCCs with basophilic or clear-cell phenotypes developed more frequently in c-myc/TGF-α and TGF-α mice and often showed a reduction or loss of β-catenin immunoreactivity. β-catenin mutation was associated with a more benign phenotype. Calvisi et al. (2004a) note that the relationship between β-catenin activation, tumor grade, and clinical outcome in human HCC remains controversial.

> There are studies that show a significant correlation between β-catenin nuclear accumulation, a high grade of HCC tumor differentiation, and a better prognosis, whereas others find that nuclear accumulation of β-catenin may be associated with poor survival or that it does not affect clinical outcome.

Calvisi et al. (2004b) report for E-cadherin a variety of morphologenetic events, including cell migration, separation, and formation of boundaries between cell layers and differentiation of each cell layer into functionally distinct structures. Loss of expression of E-cadherin was reported to result in dedifferentiation, invasiveness, lymph node or distant metastasis in a variety of human neoplasms including HCC and that the role of E-cadherin might be more complex that previously believed.

> In order to elucidate the role of E-cadherin in the sequential steps of liver carcinogenesis, we have analyzed the expression patterns of E-cadherin in a collection of preneoplastic and neoplastic liver lesions from c-Myc, E2F1, c-Myc/TGF-α and c-Myc/E2F1 transgenic mice. In particular, we have investigated the relevance of genetic, epigenetic, and transcriptional mechanisms on E-cadherin protein expression levels. Our data indicate that loss of E-cadherin contributes to HCC progression in c-Myc transgenic mice by promoting cell proliferation and angiogenesis, presumably through the upregulation of HIF-1α and VEGF proteins.

The c-Myc line, was most like wild-type and lost E-cadherin in the tumors. c-Myc/TGF-α dysplastic lesion were reported to show overexpression of E-cadherin mainly in pericentral areas with E2F1 clear cell carcinoma showed intense staining of E-cadherin. Reduction or loss of E-cadherin expression is primarily determined by loss of heterozygosity at the E-cadherin locus or by its promoter hypermethylation in human HCC Calvisi et al. (2004b) determined the status of the E-cadherin locus and promoter methylation in wild-type livers and tumors from transgenic mice by microsatellite analysis and methylation specific PCR, respectively.

> Wild-type livers and HCCs, regardless of their origins, showed the absence of LOH at the E-cadherin locus. E-cadherin promoter was not hypermethylated in wild-type, c-Myc/TGF-α and E2F1 livers. No E-cadherin promoter hypermethylation was detected in c-Myc and c-Myc/E2F1 HCCs with normal levels of E-cadherin protein. In striking contrast, seven of 20 (35%) of c-Myc and two of four (50%) c-Myc/E2F1 HCCs with downregulation of E-cadherin displayed E-cadherin promoter hypermethylation. These results suggest that promoter hypermethylation might be responsible for E-cadherin downregulation in a subset of c-Myc and c-Myc/E2F1 HCCs…The molecular mechanisms underlying down-regulation of E-cadherin in c-Myc tumors remain poorly understood at present. No LOH at the E-cadherin locus was detected in the c-Myc HCCs whereas only a subset of c-Myc tumors displayed hypermethylation of the E-cadherin promoter. Furthermore, no association was detected between E-cadherin downregulation and protein levels of transcriptional repressors, Snail, Slug or the tumor suppressor WT1, in disagreement with the finding that overexpression of Snail suppresses E-cadherin in human HCC…E-cadherin might play different and apparently opposite roles, which depend on specific tumor requirements in both human and murine liver carcinogenesis.

Importantly, the results of Calvisi et al. (2004b) show that hypermethylation of promoters can be associated with down regulation of a gene in mouse liver tumors similar to human HCC and that tumors can have the same behavior with methylation change as with loss of heterozygosity.

This report also gives evidence of the usefulness of the mouse model to study human liver cancer as it shows the similarity of dysfunctional regulation in mouse and human cancer and the heterogeneity within and between mouse lines tumors with differing dysfunctions in gene expression. This parallels human cancer where there is heterogeneity in tumors from one person and every tumor has its own signature. Finally, this report correlates differing pathway perturbations with mouse liver phenotypes similar to those reported in experimental carcinogenesis models and for TCE and its metabolites.

Farazi and DePinho (2006) suggest that

> as comparative array CGH analysis of various murine cancers has shown that such aberrations often target syntenic loci in the analogous human cancer type, we further suggest that comparative genomic analysis of available mouse model of mouse HCC might be particularly helpful in filtering through the complex human cancer genome. Ultimately, mouse models that share features with human HCCs could serve as valuable tools for gene identification and drug development. However, one needs to keep in mind key differences between mice and humans. For example, as noted in certain human HCC cases, telomere shortening might drive the genomic instability that enables the accumulation of cancer-relevant changes for hepatocarcinogenesis. As mice have long telomeres, this aspect of hepatocarcinogenesis might be fundamentally different between the species and

provide additional opportunities for model refinement and testing of this mechanism through use of a telomere deficient mouse model. These and other cross-species difference, and limitations in the use of human cell-culture systems, must be considered in any interpretation of data from various model systems (Farazi and DePinho, 2006).

Thus, these mouse models of liver cancer inductions are qualitatively able to mimic human liver cancer and support the usefulness of mouse models of cancer.

E.3.3. Hypothesized Key Events in HCC Using Animal Models

E.3.3.1. *Changes in Ploidy*

As stated above in Section E.1.1, increased polyploidization has been associated with numerous types of liver injury and appears to result from exposure to TCE and its metabolites as well as changes in the number of binucleate cells. Hortelano et al. (1995) reported that cytokines and NO can affect ploidy and further suggests a role of these changes for carcinogenesis in general. Vickers and Lucier (1996) noted that while both DEN and 17 α-ethinylestradiol have been reported to enhance the proportion of diploid hepatocytes, initiators like *N*-nitrosomorpholine are reported to increase the proportion of hypertrophied and polyploidy hepatocytes. The relationship of such changes to cancer induction has been studied in transgenic mouse models and in models involved with mitogens of differing natures.

Melchiorri et al. (1993) report the response pattern of the liver to acute treatment with primary mitogens in regard to ploidy changes occurring in rat liver following two different types of cell proliferation: compensatory regeneration induced by surgical partial hepatectomy (PH) and direct hyperplasia induced by the mitogens lead nitrate and Nafenopin (a PPARα agonist) in 8 week old male Wistar rats. Feulgen stain was used and DNA content quantified by image cytometry in mononucleate and binucleate cells. Mitotic index was determined in the same samples. The term "diploid" was used to identify cells with a single, diploid nucleus and tetraploid for cells containing 2 diploid nuclei or one tetraploid nucleus referred (bi- and mononucleate, respectively). Octoploid cells were identified as either binucleate or mononucleate.

> During liver regeneration following surgical PH an increase in the mitotic index with a peak at 24 hours was observed. The most striking effect associated with the regenerative response was the almost complete disappearance of binucleate cells, tetraploid (2 X 2c) as well as octoploid (4 X 2c) with only < 10% of the control values being present 3 days after PH…Concomitantly, an increase in mononucleate tetraploid (4c) as well as mononucleate octoploid (8c) cells was

observed, resulting at 3 days after PH in a population made up of almost entirely (98%) by mononucleated cells.

However, lead nitrate treatment was reported to induce rapid increase in the formation of binucleate cells occurring 3 days after treatment, their number accounting for 40% of the total cell population versus 22% binucleate cells in control rats and 2% in PH animals killed at the same time point. The increased binuclearity was reported to be observed only in the 4 X 2c cells (25 vs. 6% of the controls) and in 8 X 2c cells (3.7 vs. 0.1% of controls). The increase in 4 X 2c and 8 X 2c cells was reported to be accompanied by a concomitant reduction in 2 X 2c cells with the change induced in cellular ploidy by lead nitrate resulting in 37% of cells being either 8c or 16c. However, at the same time point, cells having a ploidy higher than 4c were reported to account for only 11% in PH rats and 9% in control animals. Changes in the ploidy pattern were reported to be preceded by an increased mitotic activity, which was maximal 48 hours after treatment with lead nitrate. The increase in mitotic index in lead nitrate-treated rats was associated with a striking increase in the labeling index of hepatocytes (60.1 vs. 3% of control rats) and to an almost doubling of hepatic DNA content in 3 days after lead nitrate. Melchiorri et al. (1993) concluded that the entire cell cycle appeared to be induced by lead nitrate but that the finding of a high increase of binucleate cells suggested that lead nitrate-induced liver growth, unlike liver regeneration induced by partial hepatectomy, was characterized by an uncoupling between cell cycle and cytokinesis. This raised questions whether lead nitrate-induced liver growth resulted in a true increase in cell number or is only the expression of an increased hepatocyte ploidy. They reported that part of the increase in DNA content observed 3 days after lead nitrate was indeed expression of polyploidizing process due to acytokinetic mitoses but that a consistent increase in cells number (+26%) was also induced by lead nitrate treatment.

After Nafenopin treatment, Melchiorri et al. (1993) reported that the increase in DNA content was increased 22% over controls and was much lower than induced by lead nitrate and that Nafenopin did not induce significant changes in binucleate cell number. However, a shift towards a higher ploidy class (8c) was reported to be observed following Nafenopin and the 21% increase in DNA content seen after Nafenopin treatment was almost entirely due to increase in the ploidy state with only 7% increase in cell number.

Melchiorri et al. (1993) examined whether hepatocytes characterized by high ploidy content (highly differentiated cells) would be preferentially eliminated by apoptosis. An increase in apoptotic bodies was reported to be associated with the regression phase after lead nitrate treatment (when liver mass is reduced) but despite the elimination of excess DNA, the changes in ploidy distribution induced by lead nitrate were found to persist suggested that polyploidy cells were not preferentially eliminated by apoptosis during the regression phase of the liver.

Melchiorri et al. (1993) note that other studies in rat exposed to the mitogens cyproterone acetate (CPA) and the peroxisome proliferator MCP also reported a very strong decline in binucleate cells with a concomitant increase in mononucleate tetraploid cells in the liver similar to the pattern described after partial hepatectomy.

Lalwani et al. (1997) reported the results of 1,000 ppm WY-14,643 exposure in male Wistar rats after 1, 2, and 4 weeks and suggested that an early wave of nuclear division occurred at the early stages of exposure without cumulative effects on cell proliferation. Consistent with hepatomegaly, WY-14,643-treated were reported to exhibit multifocal hepatocellular hypertrophy and karyomegaly by routine microscopic analysis. For binucleate hepatocytes, there were no reported differences between WY-14,643 and controls for days 4 and 11 but an increase in the number at Day 25 in WY-14,643-treated animals compared to controls. Increases in the diameter of nuclei were shown by WY-14,643 treatment from Day 11 and 25 with increasing numbers of cells displaying larger nuclear diameters. The mitotic index was reported not to be significantly changed in WY-14,643 treated rats compared to controls. Mitotic figures did not appear to survive the treatment necessary for flow cytometric analyses. PCNA was increased on Day 4 in WY-14,643- treated animals compared to controls whereas no differences were found on days 11 and 25. However, immunohistochemistry was reported to show remarkable increases in BrdU-labeled nuclei in liver sections after 4 days of labeling with the populations of BrdU-labeled cell declining over the course of treatment. The labeling index was high and approximately 80% of the BrdU-labeled cells were in periportal areas. PCNA-expressing cells were increased in the periportal area of the liver. Intense nuclear staining of PCNA was evident as an indicator of DNA replication in S phase. Microscopic examination showed BrdU labeling only in periportal hepatocytes, whereas no significant labeling was observed in nonparenchymal cells, indicating that the replicative activity was confined to the liver cells. Lalwani et al. (1997) suggested that their results showed that events related to cell proliferation occur in the initial phase of WY-14,643 treatment in rats but not followed by changes in the rate of DNA synthesis as the treatment progressed. They note that Marsman et al. (1988) observed constant increases in DNA synthesis by [^{3}H]-thymidine authoradiography with up to 1 year of continuous administration of WY-14,643, whereas the rate of DNA synthesis or the BrdU labeling index in their study declined after the first 4 weeks of treatment. They suggest that the increased percentage of cells appearing in G2-M phase and the analysis of liver nuclear profiles suggest that the progression of these additional cells (i.e., cells that are stimulated to enter the cell cycle by the test agent) through the cell cycle is arrested in the late stages of the cell cycle. They state

> Unlike BrdU labeling, which demonstrated DNA synthesis activity over the 4-day labeling period, the PCNA labeling index represents levels of the protein product at an interval post treatment. PCNA expression in cells exposed to chemicals or to WY may not provide true representation of S phase or proliferative activity because PCNA-expressing nuclei were also found in G0=G1 and G2-M phases.

Lalwani et al. (1997) concluded that cell proliferation alone does not appear to constitute a determining process leading to tumors in most tissues and sustained cell replication may not be a primary feature of peroxisome proliferator-induced hepatocarcinogenesis. Miller et al. (1996) note that studies with MCP in Alpk:AP rats indicate that DNA synthesis occurs primarily in one hepatocyte subpopulation as defined by ploidy status, the binucleated tetraploid (2 X 2N) hepatocytes and that this preferential hepatocyte DNA synthesis is manifested by dramatic alterations in hepatocyte ploidy subclasses, i.e., significant increases in mononucleate tetraploid (4N) hepatocytes concomitant with decreases in 2 X 2N hepatocytes. They reported results in male Fischer 344 rats were 13 weeks old (an agent in which polyploidization had reached a plateau) exposed to 1,000 ppm WY-14,643 and MCP (gavage via corn oil at 8 mg/mL or 25 mg/kg MCP once daily) for 2, 5, and 10 days ($n = 4$). WY-14,643 and MCP were reported to induce significant increases in the octoploid hepatocyte class that coincided with decreases in the tetraploid hepatocyte class. However, MCP did not induce this shift until Day 5 of exposure. These results show an approximate doubling of mononuclear octoploid (8N) hepatoctyes but still a very low number of the total hepatocyte population that does not reach greater than 7% and is still only approximately twice that of control values and thus, does not present itself with a very large target population. There was no real effect on 4N hepatocytes due to these treatments and the percent of hepatocytes that were 4N stayed ~70% and were thus, the majority cell type in the liver. Miller et al. (1996) note the importance of maturation and/or strain for these analyses there are maturation-dependent differences in the distribution and mitogenic sensitivity of hepatoctyes in the various subclasses.

Hasmall and Roberts (2000) note that despite their differing abilities to induced liver cancer, both DCB (a nonhepatocarcinogen in Fischer 344 rats) and DEHP, at the doses and routes used in the NTP bioassays, induced similar profiles of S-phase LI. A large and rapid peak during the first 7 days (1,115 and 1,151% of control for DEHP and DCB, respectively) was followed by a return to control levels. They suggest that the size of the S-phase response does not necessarily determine hepatocarcinogenic risk and that the subpopulation in which S-phase is induced may be a better correlate with subsequent hepatocarcinogenecity. They compared the effects on polyploidy/nuclearity and on the distribution of S-phase labeled cells with ETU, the peroxisome proliferator MCP, and phenobarbitone. Male F334 rats 7−9 weeks old were exposed to MCP (0.1% in diet), ETU 83 ppm diet, phenobarbitone (500 mg/mL drinking water) for 7 days. The number of rats for 7 day study was not given by the authors. Hasmall and Roberts (2000) reported that treatment of rats with MCP, ETU or phenobarbitone for 7 days had no significant effect on the ploidy profile as compared with corn oil controls (data not shown) but that MCP and phenobarbitone did induce significant changes in nuclearity. MCP reduced the 2 X 2N population and increased the 8N population. Phenobarbitone similarly increased the proportion of cells in the 4N population. ETU had no effect on the nuclearity profile as compared with control. However, what the authors describe for their results in polidy and nuclearity are different than those presented in their figures. There were significant differences between controls that the authors did not characterize and there appeared to be a greater difference between controls than some of the treatments.

Gupta (2000) report that in transgenic mice with overexpression of TGF-α, liver-cell turnover increases, along with the onset of hepatic polyploidy, whereas hepatocellular carcinoma originating in these animals contain more diploid cells. They note that coexpression of c-Myc and TGF-α transgenes in mouse hepatocytes was associated with greater degrees of polyploidy as well as increased development of hepatocellular carcinoma. Gupta (2000) notes that in the presence of ongoing liver injury and continuous depletion of parenchymal cells, hepatic progenitor cells (including oval cells) are eventually activated but what roles polyploid cells play in this process requires further study. In the working model by Gupta (2000), sustained disease by chronic hepatitis, metabolic disease, toxins, etc., may lead to hepatocyte polyploidy and loss, and the emergence of rapidly cycling progenitor or escape cell clones with the onset of liver cancer.

Conner et al. (2003) describe the development of transgenic mouse models in which E2F1 and/or c-Myc was overexpressed in mouse liver. The E2F1 and c-Myc transcription factors are both involved in regulating key cellular activities including growth and death and, when overexpressed, are capable of driving quiescent cells into S-phase in the absence of other mitogenic stimuli and are potent inducers of apoptosis operating at least through one common pathway involving p53. Deregulation of their expression is also frequently found in cancer cells (Conner et al., 2003). Conner et al. (2003) reported that although both c-Myc and E2F1 mono-transgenic mice were prone to liver cancer, E2F1 mice developed HCC more rapidly and with a higher frequency and that the combined expression of these two transcription factors dramatically accelerated HCC growth compared to either E2F1 or c-Myc mono-transgenic mice. All three transgenic lines were reported to show a low but persistent elevation of hepatocyte proliferation before an onset of tumor growth. Ploidy was shown to be affected differently by c-Myc and E2F1, and suggested distinct differences by which these two transcription factors control liver proliferation/maturation. Both transgenic alterations induced liver cancer but had differing effects on polyploidization suggestive that liver cancer can arise from either type of mature hepatocyte.

c-Myc single-transgenic mouse showed a continuous high cell proliferation that preceded the appearance of preneoplastic lesions, which was also true, although to a lesser extent, in the E2F1 mouse. At 15 weeks of age, all of the transgenic mouse lines were reported to have a high incidence (>60%) of hepatic dysplasia with mitotic indices equivalent in c-Myc/E2F1, and c-Myc livers, but 2-fold higher than the mitotic index in E2F1 and very low in wild-type mice. Thus, the combination of the two transgenes did not have an additive effect on proliferation. An analysis of the DNA content in hepatocyte nuclei isolated from 4- to 15-week old mice was reported to show that in young wild-type livers, the majority of nuclei had a diploid DNA content with a smaller proportion of tetraploid nuclei. As the mice aged, the number of tetraploid and octoploid nuclei increased consistent with the previous findings of others. However, c-Myc mice were reported to demonstrate a premature polyploidization with the number of 2N nuclei in c-Myc livers almost 2-fold less, while the proportion of 4N nuclei increased more than 2.5-fold at 4 weeks of age. The most prominent ploidy alteration was an increase in the fraction of hepatocytes with octaploid nuclei (~200-fold higher). The percentage of polyploidy cells was reported to continue to rise in 15 week old c-Myc livers. The majority of hepatocytes had nuclei with 4N and 8N DNA content, with an attendant increase in binucleated hepatocytes and increase in average cell size. In striking contrast, E2F1 hepatocytes were reported not to undergo normal polyploidization with aging. The majority of E2F1 nuclei were reported to remain in the diploid state and to be almost identical in E2F1 mice at 4 and 15 weeks of age. The percentage of binucleated hepatocytes was also reduced. In c-Myc/E2F1 mice, the age-related changes in ploidy distribution were reported to resemble those found in both c-Myc and in E2F1 single transgenic mice. At a young age, c-Myc/E2F1 mice, similar to E2F1 mice, were reported to retain significantly more diploid nuclei than c-Myc mice. However, as mice aged, the majority of c-Myc/E2F1 hepatocytes, similar to c-Myc cells but in contrast to findings in E2F1 cells, became polyploid. Consistent with a more progressive polyploidization, the DNA content was significantly higher in both c-Myc/E2F1 and c-Myc livers. Conner et al. (2003) report that other known modulators of ploidy in the liver are the tumor suppressor p53, pRb, and the cell cycle inhibitor p21 as well as, genes involved in the control of the cell cycle progression such as cyclin A, cyclin B, cyclin D3, and cyclin E.

Along with increased liver cancer, Conner et al. (2003) note that the C-Myc mice also experienced a persistent liver injury as evidenced by significant elevation of circulating levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase along with the appearance of a frequent oval/ductular proliferation. However, oval cell proliferation may be a marker of hepatocyte damage but not be the cells responsible for tumor induction (Tarsetti et al., 1993). Conner et al. (2000) report that if E2F1 is overexpressed in the liver, there is both oncogenic and tumor-suppressive properties. In regard to liver morphological changes, E2F1 transgenic mice were reported to uniformly develop pericentral dysplasia and foci adjacent to portal tracts followed by the abrupt appearance of adenomas and subsequent malignant conversion with all of the animals having foci by 2−4 months and by 8−10 months most having adenomas with dysplastic changes remaining confined to the pericentral regions of the liver lobule. In regard to phenotype, the majority of the foci were composed of small round cells, with clear-cell phenotype but eosinophilic, mixed, and basophilic foci were also seen. In adenomas with malignant transformation to HCC, there appeared to be high mitotic indices, blood vessel invasion, and central collection of deeply basophilic cells with large nuclei giving a "nodule- in-nodule" appearance. Macrovesicular hepatic steatosis was first noted in some E2F1 transgenic livers at 6−8 months and by 10−12 months 60% of animals had developed prominent fatty change. Hepatic steatosis has been noted in several transgenic mouse models of liver carcinogenesis (Conner et al., 2000). These results raise interesting points of regional difference in tumor formation which can be lost in analyses using whole liver and that the phenotype of foci and tumors are similar to those seen from chemical carcinogenesis. The occurrence of hepatotoxicity in these transgenic mice is also of note.

E.3.3.2. *Hepatocellular Proliferation and Increased DNA Synthesis*

Caldwell et al. (2008b) have presented a discussion of the role of proliferation in cancer induction. They state that

> in the case of CCl4 exposure, hepatocyte proliferation may be related to its ability to induce liver cancer at necrogenic exposure levels, but the nature of this proliferation is fundamentally different from peroxisome proliferators or other primary mitogens that cause hepatocyte proliferation without causing cell death (Coni et al., 1993; Ledda-Columbano et al., 1993, 1998, 2003; Menegazzi et al., 1997; Columbano and Ledda-Columbano, 2003). After initiation with a mutagenic agent, the transient proliferation induced by primary mitogens has not been shown to lead to cancer-induction, while partial hepatectomy or necrogenic treatments of CCl4 result in the development of tumors [Ledda-Columbano et al., 1993; Gelderblom et al., 2001].

Roskams et al. (2003) notes that partial hepatectomy does not cause hepatocellular carcinoma in normal mice without initiation. Melchiorri et al. (1993) report that a series of studies has shown that acute proliferative stimuli provided by primary mitogens, unlike those of the regenerative type such as those elicited by surgical or chemical partial hepatectomy, do not support the initiation phase and do not effectively promote the growth of initiated cells (Columbano et al., 1990; Columbano et al., 1987; Ledda-Columbano et al., 1989). They note that, the finding that most of these chemicals, with the exception of WY, induce only a very transient increase in cell proliferation raises the question whether such a transient induction of liver cell proliferation might be related to liver cancer appearing 1−2 years later. They note that mitogen-induced liver growth differs from compensatory regeneration in several aspects (1) it does not require an increased expression of hepatocyte growth factor mRNA in the liver (2) it is not necessarily associated with an immediate early genes such as c-fos and c-jun; (3) it results in an excess of tissue and hepatic DNA content that is rapidly eliminated by apoptotic cell death following withdrawals of the stimulus.

Other studies have questioned the importance of a brief wave of DNA synthesis in induction of liver cancer. Chen et al. (1995) note that Jirtle et al. (1991) and Schulte-Hermann et al. (1986) reported that during a 2-week period of treatment with lead, DNA synthesis was increased most in centrolobular hepatocytes and that the predominantly centrilobular distribution of the labeled nuclei may have been due largely to the brief wave of mitogenic response, because from the fifth day onward DNA synthesis activity returned to control level even though lead nitrate treatment continued. They concluded that sustained cell proliferation may be more important than a brief wave of increased DNA synthesis. Chen et al. (1995) also noted that a number of different agents acting via differing MOAs will induce periportal proliferation.

Vickers and Lucier (1996) reported that mitogenic response induced by acute 17 α-ethinylestradiol administration is randomly distributed throughout the hepatic lobule, while continuous administration increases the proportion of diploid cells. Richardson et al. (1986) reported that the lobular distribution of the correlation of hepatocyte initiation and akylation reported in their model of carcinogenicity did “not support that early proliferation is associated with cancer as at 7 days there is a transient increase in the lobes least likely to get a tumor and no difference between the lobes at 14 and 28 days DEN although there is a difference in tumor formation between the lobes.” Cells undergoing DNA synthesis may not be in the same zone of the liver where other hypothesized “key events” take place.

Tanaka et al. (1992) note that the distribution of hepatocyte proliferation in the periportal area was in contrast to the distribution of peroxisome proliferation in the centrilobular area of Clofibrate treated rats. Melnick et al. (1996) note that replicative DNA synthesis commonly has been evaluated by measurement of the fraction of cells incorporating BrdU or tritiated thymidine into DNA during S-phase of the cell cycle (S-phase labeling index), but that the S-phase labeling index would not be identical to the cell division rate when replication of DNA does not progress to formation of two viable daughter cells. "The general view at an international symposium on cell proliferations and chemical carcinogenesis was that although cell replication is involved inextricably in the development of cancers, chemically enhanced cell division does not reliably predict carcinogenicity (Melnick et al ,1993)." They note that the finding that enzyme-altered hepatic foci were not induced in rats fed WY-14,643 for 3 weeks followed by partial hepatectomy indicates that early high levels of replicative DNA synthesis and peroxisome proliferation are not sufficient activities for initiation of hepatocarcinogenesis. Baker et al. (2004) reported that, similar to the pattern of transient increases in DNA synthesis reported for TCE metabolites, Clofibrate exposure induced the upregulation of a variety of cell proliferation-associated genes (e.g., G2/M specific cyclin B1, cyclin-dependent kinase 1, DNA topoisomerase II alpha, c-myc protooncogene, pololike serien-threonine protein kinase, and cell divisions control protein 20) began on or before Day 1 and peaked at some point between days 3 and 7. By Day 7, cell proliferation genes were down regulated. The chronology of this gene expression agrees with the histologic diagnosis of mitotic figures in the tissue, where an increase in mitotic figures was detected in the Day 1 and most notably Day 3 high and low-dose groups. However, by Day 7, the incidence of mitotic figures had decreased. The clustering of genes associated with the G2/M transition point suggests that in the rats, the polyploid cells arrested at G2/M are those that are proceeding through the cell cycle.

A dose-response for increased DNA-synthesis also seems to be lacking for the model PPARα agonist, WY-14,643 suggesting that the transient increases in DNA synthesis reported by Eacho et al. (1991) for this compound at lower levels that then increase later at necrogenic exposure levels, are not related to its carcinogenic potential. Wada et al. (1992) reported that in male Fischer 344 rats exposed to a range of WY-14,643 concentrations (5−1,000 ppm) that liver weight gain occurred at the lowest dose that gave a sustained response for many weeks but gave increased cell labeling only in the first week. Peroxisomes proliferation, as measure by electron microscopy, increases started at 50 ppm exposures. By enzymatic means, peroxisomal activities were elevated at the 5 ppm dose. Of note is the reported difference in distribution in hepatocellular proliferation, which was not where the hypertrophy or where the lipofuscin increases were observed. The authors note that these data suggest that 50 and 1,000 ppm WY-14,643 should give the same carcinogenicity if peroxisome proliferation or sustained proliferation are the "key events." The study of Marsman et al. (1992) is very important in that it not only shows that clofibric acid (another PPAR α agonist) does not have sustained proliferation, but it also shows that it and WY-14,643 at 50 ppm did not induce apoptosis in rats. It is probable that use of WY-14,643 at high concentrations may induce apoptosis in a manner not applicable to other peroxisome proliferators or to treatment with WY-14,643 at 50 ppm. This study also confirmed that exposure to WY-14,643 at 50 ppm and WY-14,643 at 1,000 ppm induces similar effects in regards to hepatocyte proliferation and peroxisomal proliferation.

The study by Eacho et al. (1991) also gives a reference point for the degree of hepatocytes undergoing transient DNA synthesis from WY-14,643 and Clofibrate and how much smaller it is for TCE and its metabolites, which generally involve less than 1% of hepatocytes.

> The labeling index of BrdU was 7.2% on day 3 and 15.5% on day 6 after clofibric acid but by day 10 and 30 labeling index was the same as controls at ~1-2%.…For WY the labeling index was 34.1% at day 3 and 18.6% at day 6. At day 10 the labeling index was 3.3% and at day 30 was 6%, representing 6.6- and 15-fold of respective controls. Control levels were ~0.5 to 1%.…The labeling index was increased to 32% by 0.3% LY171883 and to 52% by 0.05% Nafenopin. The 0.005% and 0.1% dietary doses of WY increased the 7 day labeling index to a comparable level (55% - 58%).

Yeldani et al. (1989) report results showing that until foci appear, cell proliferation has ceased to increase over controls after the first week for ciprofibrate-induced hepatocarcinogenesis. The results also show the importance of using age matched controls and not pooled controls for comparative purposes of proliferation as well as how low proliferative rates are in control animals. The results of Barass et al. (1993) are important in suggesting that age of animals is important when doing quantitation of labeling indexes. Studies such as that conducted by Pogribny et al. (2007) that only give the replication rate as a ratio to control will make the proliferation levels look progressive when in fact they are more stable with time as it is just the controls that change with age as a comparison point.

E.3.3.3. *Nonparenchymal Cell Involvement in Disease States Including Cancer*

The recognition that not only parenchymal cells but also nonparenchymal cells play a role in HCC has resulted in studies of their role in initiation as well as progression of neoplasia. The role of the endothelial cell in controlling angiogenesis, a prerequisite for neoplastic progression, and the role of the Kupffer cell and its regulation of the cytokine milieu that controls many hepatocyte functions and responses have been reported. However, as pointed out by Pikarsky et al. (2004) and by the review by Nickoloff et al. (2005) the roles of inflammatory cytokines in cancer are context and timing specific and not simple. For TCE, nonparenchymal cell proliferation has been observed after inhalation (Kjellstrand et al., 1983b) and gavage (Goel et al., 1992) exposures of ~4 weeks duration.

E.3.3.3.1. *Epithelial cell control of liver size and cancer—angiogenesis.*

The epithelium is key in controlling restoration after partial hepatectomy and not surprisingly HCC growth. Greene et al. (2003) hypothesized that the control of physiologic organ mass was similar to the control of tumor mass in the liver and that specifically, the proliferation of hepatocytes after partial hepatectomy, like the proliferations of neoplastic cells in tumors, requires the synthesis of new blood vessels to support the rapidly increasing mass. They report that a peak in hepatocyte production of vascular endothelial growth factor (VEGF), an endothelial mitogen, corresponds to an increase of VEGF receptor expression on endothelial cells after partial hepatectomy and the rate of endothelial proliferation.. Fibroblast growth factor and transforming growth factor-alpha (TGfox), which stimulate endothelial cells, are secreted by hepatoctyes 24 hours after partial hepatectomy. However, endothelial cells were reported to secrete hepatocyte growth factor, a potent hepatocyte mitogen, that is also proangiogenic. The secretion of transforming growth factor –beta by (TGfox) endothelial cells 72 hours after partial hepatectomy was reported to inhibit hepatocyte proliferation. Thus, Greene et al. (2003) suggested that endothelial cells and hepatocytes of the regenerating liver influence each other, and both populations are required for the regulation of the regenerative process.

E.3.3.3.2. *Kupffer cell control of proliferation and cell signals, role in early and late effects*

Vickers and Lucier (1996) have reported that Kupffer cells are increased in number in prenoplastic foci but are decreased in hepatocellular carcinoma, and that other studies have demonstrated that both sinusoidal endothelial cells and Kupffer cells within hepatocellular carcinoma cells in humans stain positive for mitotic activity although the number of nonparenchymal cells compared to parenchymal cells may be reduced. Lapis et al. (1995) reported that Kupffer cells contain lysozyme in their cytoplasmic granules, vacuoles and phagosomes, some cells show a positive reaction in the rough endoplasmic reticulum, perinuclear cisternae and the Golgi zone, and that in human monocytes the lysozyme is colocalized with the CD68 antigen and myeloperoxidase. They also report that, in rodent hepatocarcinogenesis, increased numbers of Kupffer cells were observed in preneoplastic foci, whereas abnormally low numbers were present following progression to hepatocellular carcinoma. They also note that "the Kupffer cell count in human HCC has also been shown to be very low and varies with different histological form." They reported that for monkey HCCs, that the proportion of endothelial elements remained constant (the parenchymal/endothelial cell ratio), however, there was a striking reduction in the areas occupied by Kupffer cells. While healthy control livers contained the highest number of Kupffer cells, in the tumor-bearing cases the nonneoplastic, noncirrhotic liver adjacent to the HCC nodules had a significantly lower number of Kupffer cells and the number decreased further in the nonneoplastic portions of cirrhotic livers. Within HCC nodules the Kupffer cell count was greatly reduced with no significant changes were observed between the cirrhotic areas and the carcinomas, however, the tumors contained fewer lysozyme and CD68 positive cells. Lapis et al. (1995) note that

> since other cell types within the liver sinusoids (monocytes and polypmorphs) and portal macrophage were also positive, it was important to identify the star-like morphology of the Kupffer cells. The results of the two independent observers assessment of the morphology and enumeration of Kupffer cells were quite consistent and differed by only 3%." "The loss of Kupffer cells in the HCC may possibly result from capillarization of the sinusoids, which has been observed during the process of liver cirrhosis and carcinogenesis. Capillarization entails the sinusoidal lining endothelial cells losing their fenestrations.

E.3.3.3.3. *Nf-kB and TNF-α - context, timing and source of cell signaling molecules*

A large body of literature has been devoted to the study of nuclear factor κ B for its role not only in inflammation and a large number of other processes, but also for its role in carcinogenesis. However, the effects of these cytokines are very much dependent on their cellular context and the timing of their modulation. As described by Adli and Baldwin (2006),

> The classic form of NF-kB is composed of a heterodimer of the p50 and p65 subunits, which is preferentially localized in the cytoplasm as an inactive complex with inhibitor proteins of the IkB family. Following exposure to a variety of stimuli, including inflammatory cytokines and LPS, IkBs are phosphorylated by the IKKα/β complexes then accumulate in the nucleus, where they transcriptionally regulate the expression of genes involved in immune and inflammatory responses.

The five members of the mammalian NF-kB family, p65 (RelA), RelB, c-Rel, P50/p105 (NF-KB1) and p52/p100 (NF-kB2), exist in unstimulated cells as homo- or heterodimers bound to IkB family proteins. Transcriptional specificity is partially regulated by the ability of specific NF-kB dimmers to preferentially associate with certain members of the IkB family. Individual NF-kB responses can be characterized as consisting of waves of activation and inactivation of the various NF-kB members (Hayden and Ghosh, 2004). While the function of NF-kB in many contexts have been established, it is also clear that there is great diversity in the effects and consequences of NF-kB activation with NF-kB subunits not necessarily regulating the same genes in an identical manner and in all of the different circumstances in which they are induced. The context within which NF-kB is activated, be it the cell type or the other stimuli to which the cell is exposed, is therefore, a critical determinant of the NF-kB behavior (Perkins and Gilmore, 2006).

Balkwill et al. (2005) report that

> the NF-κB pathway has dual actions in tumor promotion: first by preventing cell death of cells with malignant potential, and second by stimulating production of proinflammatory cytokines in cells of infiltrating myeloid and lymphoid cells. The proinflammatory cytokines signal to initiated and/or otherwise damaged epithelial cells to promote neoplastic cell proliferation and enhance cell survival. However, the tumor promoting role of NF-κB may not always predominate. In some cases, especially early cancers, activation of this pathway may be tumor suppressive (Perkins, 2004). Inhibiting NF-κB in keratinocytes promotes squamous cell carcinogenesis by reducing growth arrest and terminal differentiation of initiated keratinocytes (Seitz et al., 1998).

Other inflammatory mediators have also been associated with oncogenesis. Balkwill et al. (2005) reported that TNFα is frequently detected in human cancers (produced by epithelial tumor cells, as in for instance, ovarian and renal cancer) or stromal cells (as in breast cancer). They also report that the loss of hormonal regulation of IL-6 is implicated in the pathogenesis of several chronic diseases, including B cell malignancies, renal cell carcinoma, and prostate, breast, lung, colon, and ovarian cancers. Over 100 agents, such as antioxidants, proteosome inhibitors, NSAIDs, and immunosuppressive agents are NF-κB inhibitors with none being entirely specific (Balkwill et al., 2005). Thus, alterations in these cytokines, and the cells that produce them, are implicated as features of "cancer" rather than specific to HCC.

Balkwill et al. (2005) report that

> Two mouse models of inflammation-associated cancer now implicate the gene transcription factor NF-κB and the inflammatory mediator known as tumor-necrosis factor α (TNF- α) in cancer progression. Using a mouse model of inflammatory hepatitis that predisposes mice to liver cancers, Pikarsky et al. present evidence that the survival of hepatocytes - liver cells - and their progression to malignancy are regulated by NF-κB. NF-κB is an important transcription factor that controls cell survival by regulating programmed cell death, proliferation, and growth arrest. Pikarsky et al. find that the activation state of NF-κB, and its localization in the cell, can be controlled by TNF-α produced by neighboring inflammatory cells (collectively known as stromal cells).

Pikarsky et al. (2004) reported that that the inflammatory process triggers hepatocyte NF-κB through upregulation of TNF-α in adjacent endothelial and inflammatory cells. Switching off NF-κB in mice from birth to seven months of age, using hepatocyte-specific inducible IκB-super repressor transgene, had no effect on the course of hepatitis, nor did it affect early phases of hepatocyte transformation. By contrast, suppressing NF-κB inhibition through anti-TNF-α treatment or induction of the IκB-super repressor in later stages of tumor development resulted in apoptosis of transformed hepatocytes and failure to progress to hepatocellular carcinoma. The Mdr2 knockout hepatocytes in Pikarsky's model of hepatocarcinogenicity were distinguishable from wild-type cells by several abnormal features; high proliferation rate, accelerated hyperploidy and dysplasia. Pikarsky et al. (2004) reported that NF-κB knockout and double mutant mice displayed comparable degrees of proliferation, hyperploidy and dysplasia implying that NF-κB is not required for early neoplastic events. Thus, activation of NF-κB was not important in the early stages of tumor development, but was crucial for malignant conversion.

> Greten et al reporting in Cell, come to a similar conclusion by studying a mouse colitis-associated cancer model. Their work does not directly implicate TNF-α, but instead found enhanced production of several pro-inflammatory mediators (cytokines) including TNF-α,, in the tumor microenvironment during the development of cancer. An important feature of both studies is that NF-κB activation was selectively ablated in different cell compartments in developing tumor masses, and at different stages of cancer development.

Balkwill et al. (2005) also note that TNF-α and NF-κB have many different effects, depending on the context in which they are called into play and the cell type and environment.

In contrast, El-Serag and Rudolph (2007) note that "the influence of inflammatory signaling on hepatocarcinogenesis can be context dependent; deletion of Nf-κB-dependent inflammatory responses enhanced HCC formation in carcinogen treated mice (Sakurai et al., 2006)." Similarly, deletion of Nf-κB essential modulator/I kappa β kinase (NEMO/IKK), an activator of Nf-κB, induced steatohepatitis and HCC in mice (Luedde et al., 2007). Maeda et al. (2005) reported that hepatocyte specific deletion of IKKβ (which prevents NF-kB activation) increased DEN-induced hepatocarcinogenesis and that a deletion of IKKβ in both hepatocytes and hematopoietic-derived cells, however, had the opposite effect, decreasing compensatory proliferation and carcinogenesis. They suggest that these results, differ from previous suggestion that the tumor-promoting function of NF-kB is excreted in hepatocytes (Pikarsky et al., 2004), and suggest that chemicals or viruses that interfere with NF-kB activation in hepatocytes may promote HCC development.

Alterations in NF-kB levels have been suggested as a key event for the hepatocarcinogenicity by PPARα agonists. The event associated with PPAR effects has been the extent of NF-kB activation as determined through DNA binding. As reported by Tharappel et al. (2001), NF-kB activity is assayed with electrophoretic modibility shift assay with nuclear extracts prepared from frozen liver tissue as a measure of DNA binding of NF-kB. Increase transcription of downstream targets of NF-kB activity have also been measured. It has been suggested that PPARα may act as a protective mechanism against liver toxicity. Ito et al. (2007) cite repression of NF-kB by PPARα to be the rationale for their hypothesis that PPARα-null mice may be more vulnerable to tumorigenesis induced by exposure to environmental carcinogens. However, as shown in Section E.3.4.1.2, although DEHP was reported to also induce glomerularnephritis more often in PPARα-null mice, as suggested Kamijo et al. (2007) to be due of the absence of PPARα- dependent anti-inflammatory effect of antagonizing the oxidative stress and NF-κB pathway, there was no greater or lesser susceptibility to DEHP-induced liver carcinogenicity in the PPARα null mice.

Because PPARα is known to exert anti-inflammatory effects by inducing expression of IκBα, which antagonizes NFκB signaling, the expression of IκBα has been measured in some studies (Kamijo et al., 2007) as well as expression of TNR1 mRNA to evaluate the sensitivity to the inflammatory response. Ito et al. (2007) report that in wild-type mice there did not appear to be a difference between controls and DEHP treatment for p65 immunoblot results. DEHP treatment was also reported to not induce p65 or p52 mRNA either or influence the expression levels of TNFα, IkBα, IkBβ and IL-6 mRNA in wild-type mice. Tharappel et al. (2001) treated rats with WY-14,643, gemfibrozil or Dibutyl phthalate and reported elevated NF-kB DNA binding in rats with WY-14,642 to have sustained response but not others. WY-14,643 increased DNA binding activity of NF-kB at 6, 34 or 90 days. Gemfibrozil and DEHP increased NF-kB activity to a lesser extent and not at all times in rats. For gemfibrozil, there was only a 2-fold increase in binding at 6 days with no increase at 34 days and increase only in low dose at 90 days. In rats treated with Dibutyl phthalate, there no change at 6 days, at 34 days there was an increase at high and low dose, at 90 days only low dose animals showed a change. In pooled tissue from WY-14,643- treated animals, the complex that bound the radiolabeled NF-kB fragment did contain both p50 and p65. Both WY-14,643 and gemfibrozil were reported to produce tumors in rats with Dibutyl pthalate untested in rats for carcinogenicity. Thus, early changes in NF-kB were not supported as a key event and WY-14,643 to have a pattern that differed from the other PPARα agonists examined.

In regard to the links between inflammation and cancer, Nickoloff et al. (2005) in their review of the issue, caution that such a link is not simple. They note that

> dissecting the mediators of inflammation in cutaneous carcinogenic pathways has revealed key roles for prostaglandins, cyclooxygenase-2, tumor necrosis factor-α, AP-1, NF-κB, signal transducer and activator of transcription (STAT)3, and others. Several clinical conditions associated with inflammation appear to

predispose patients to increased susceptibility for skin cancer including discoid lupus erythematosus, dystrophic epidermolysis bullosa, and chronic wound sites. Despite this vast collection of data and clinical observations, however, there are several dermatological setting associated with inflammation that do not predispose to conversion to lesions into malaignancies such as psoriasis, atopic dermatitis, and Darier's disease.

Nickoloff et al. (2005) suggest that such a

> link may not be as simple as currently portrayed because certain types of inflammatory processes in skin (and possibly other tissues as well) may also serve a tumor suppressor function. Over the past few months, several publications in leading biomedical journals grappled with an important issue in oncology, namely defining potential links between chronic tissue damage, inflammation, and the development of cancer. Balkwill and Coussens (2004) reviewed the role of the NF-κB signal transduction pathway that can regulate inflammation and also promote malignancy. Their review summarized the latest findings revealed in a letter to Nature by Pikarsky et al. (2004). Using Mdr2 knockout mice in which hepatitis is followed by hepatocellular carcinoma, Pikarsky et al. implicated TNFα upregulation in tumor promotion of HCC, and suggest that TNFα and NF-κB are potential targets for cancer prevention in the context of chronic inflammation. A similar conclusion was reached with respect to NF-κB by an independent group of investigators using a model of experimental dextran sulfate-induced colitis, in which inactivation of the IκB kinase resulted in reduced colorectal tumors (Greten et al ., 2004). Although there are many other clinical condition supporting the concept of inflammation is a critical component of tumor progression (e.g., reflux esophagitis/esophageal cancer; inflammatory bowel disease/colorectal cancer), there is at least one notable example that does not fit this paradigm. As described below, psoriasis is a chronic cutaneous inflammatory disease, which is seldom if ever accompanied by cancer suggesting the relationship between tissue repair, inflammation, and development may not be as simple as portrayed by the aforementioned reviews and experimental results. Besides psoriasis, other noteworthy observations pointing to more complexity include the observation that in the Mdr2 knockout mice, we rarely detect bile duct tumors despite extensive inflammation, NF-κB activation, and abundant proliferation of bile ducts in portal spaces (Pikarsky et al., 2004). Moreover, in a skin-cancer mouse model, NF-κB was shown to inhibit tumor formation (Dajee et al., 2003). Thus, the composition of inflammatory mediators, or the properties of the responding epithelial cells (e.g., signaling machinery, metabolic status), may dictate either tumor promotion or tumor suppression. Chronic inflammation and tissue repair can trigger pro-oncogenic events, but also that tumor suppressor pathways may be upregulated at various sites of injury and chronic cytokine networking.
>
> One cannot easily dismiss the many dilemmas raised by the psoriatic plaque that confound a simple link between the tissue repair, inflammation, and

> carcinogenesis. Since it is easily visible to the naked eye, and patients may suffer from such lesions for decades, it is difficult to argue that various skin cancers such as squamous cell carcinoma, basal cell carcinoma, or melanoma actually do develop within plaques by are being overlooked by patients and dermatologists. Remarkably, psoriatic plaques are intentionally exposed to mutagenic agents including excessive sunlight, topical administration of crude coal tar, or parenteral DNA cross-linking agent –psoralen followed by ultraviolet light. Moreover these treatments are known to induce skin cancer in nonlesional skin. Thus since psoriatic skin is characterized by altered differentiation, angiogenesis, increased telomerase activity, proliferative changes, and apoptosis resistance, one would expect that each and every psoriatic plaque would be converted to cancer, or at least serve as fertile soil for the presence of non-epithelial skin cancers over time.…In conclusion, it would seem prudent to remember the paradigm proposed by Weiss (1971) in which he suggested that premalignant cells do not comprise an isolated island, but are a focus of intense tissue interactions. The myriad inflammatory effects of the tumor microenvironment are important for understanding tumor development, as well as tumor suppression and senescence, and for the design for efficacious prevention strategies against inflammation-associate cancer (Nickoloff et al., 2005).

E.3.3.4. *Gender Influences on Susceptibility*

As discussed previously, male humans and rodents are generally more likely to get HCC. The increased risk of liver tumors from estrogen supplements in women has been documented. In mice male TCE exposure has been shown to have greater variability in response and greater effects on body weight in males (Kjellstrand et al., 1983a, b) but to also induce dose-related increases in liver weight and carcinogenic response in female mice as well as males (see Section E.2.3.3.2). Recent studies have attempted to link differences in inflammatory cytokines and gender differences in susceptibility.

Lawrence et al. (2007) suggest that

> studies of Naugler et al. (2007) and Rakoff-Nahoum and Medzhitov (2007), advance our understanding of the mechanisms of cancer-related inflammation. They describe an important role for an intracellular signaling protein called MyD88 in the development of experimental liver and colon cancers in mice. MyD88 function has been well characterized in the innate immune response (Akira and Takeda, 2004), relaying signals elicited by pathogen-associated molecules and by the inflammatory cytokine interleukin-1 (IL-1).…The conclusion from Naugler et al. (2007) and Rakoff-Nahoun and Medzhitov is that MyD88 may function upstream of NF-κB in cells involved in inflammation-associated cancer. Immune cells infiltrate the microenvironment of a tumor. Naugler et al. (2007) and Rakoff-Nahoun and Medzhitov (2007) suggest that the development of liver and intestinal cancers in mice may depend on a signaling

pathway in infiltrating immune cells that involved the protein MyD88, the transcription factor NF-κB, and the pro-inflammatory cytokine Il-6. TLR binds a ligand which acts on MyD88 which acts on NF-κB which leads to secretion of inflammatory cytokine IL-6 which leads to promotion of tumor cell survival and proliferation.

Naugler et al. (2007) suggested gender disparity in MyD88–dependent IL-6 production was linked to differences in cancer susceptibility using the DEN model (a mutagen with concurrent regenerative proliferation at a single high dose) with a single injection of DEN. Partial hepatectomy was reported to induce no gender-related difference in IL-6 increase. After DEN treatment the male mouse had 275 ng/mL as the peak IL-6 levels 12 hours after DEN and for female mice the peak was reported to be 100 ng/mL 12 hours after DEN administration. This is only about a 2.5-fold difference between genders. Il-6 mRNA induction was reported for mice 4 hours after DEN while at 4 hours, at a time when there was no difference in serum IL-6 between male and female mice. It was not established that the 4-hour results in mRNA translated to the differences in serum at 12 hour between the sexes. The magnitude of mRNA differences does not necessarily hold the same relationship as the magnitude in serum protein. In fact, there was not a linear correlation between mRNA induction and IL-6 serum levels.

A number of issues complicate the interpretation of the results of the study. The study examined an acute response for the chronic endpoint of cancer and may not explain the differences in gender susceptibility for agents that do not cause necrosis. The DEN was administered in 15-day old mice (which had not reached sexual maturity) for tumor information at a much lower dose than used in short-term studies of inflammation and liver injury in which mature mice were used. If large elevations of IL-6 are the reason for liver cancer, why does not a partial hepatectomy induce liver cancer in itself? The percentage of proliferation at 36 and 48 hours after partial hepatectomy was the same between the sexes. If a 2.5-fold difference in IL-6 confers gender susceptibility, it should do so after partial hepatectomy and lead to cancer. For female mice, partial hepatectomy showed alterations in a number of parameters. However, partial hepatectomy does not cause cancer alone. The 5-fold increase 4 hours after DEN induction of IL-6 mRNA in male mice is in sharp contrast to the 27-fold induction of IL-6 1 hour after partial hepatectomy (in which at 4 hours the IL-6 had diminished to 6-fold). There appeared to be variability between experiments. For example, the difference in males between experiments appears to be the same magnitude as the difference between male and female in one experiment and the baseline of IL-6 mRNA induction appeared to be highly variable between experiments as well as absolute units of ALT in serum 24 and 48 hours after DEN treatment that tended to be greater that the effects of treatments. The experiments used very few animals

(n = 3) for most treatment groups. Of note is that the MyD88 -/- male mice still had a background level of necrosis similar to that of WT mice at 48 hours after DEN treatment, a time, long after the peak of IL-6 mRNA induction and IL-6 serum levels were reported to have peaked. One of the key issues regarding this study is whether difference in IL-6 reported here lead to an increase proliferation and does that difference within 48 hours of a necrotizing dose of a carcinogen change the susceptibility to cancer? This report shows that male and female mice have a difference in necrosis after CCL4 and a difference in proliferation. Are early differences in IL-6 at 4 hours related to the same kind of stimulus that leads to necrosis and concurrent proliferation? The amount of proliferation (as measured by DNA synthesis) between male and female mice 48 hours after DEN was very small and the study was conducted in a very few mice (n = 3). At 36 hours the degree of proliferation was almost the same between the genders and about 0.6% of cells. The baseline of proliferation also differed between genders but the variation and small number of animals made it insignificant statistically. At 48 hours the differences in proliferation between male and female mouse were more pronounced but still quite low (2% for males and ~1% for females). Is the change in proliferation just a change in damage by the agent? Given the large variation in serum ALT and by inference necrosis, is there an equal amount of variability in proliferation? This study gives only limited information for DEN treatment.

The difference in incidence of HCC was reported to be greater than that of "proliferation" between genders and of other parameters although differences in tumor multiplicity or size between the genders are never given in the paper. Most importantly, comparisons between the short-term changes in cytokines and indices of acute damage are for adult animals that are sexually mature and at doses that are 4 times (100 vs. 25 mg/kg) that of the sexually immature animals who are going through a period of rapid hepatocyte proliferation (15 day old animals). It is therefore, difficult to extrapolate between the two paradigms to distinguish the effects of hormones and gender on the response. Finally, the work of Rakoff-Nahoum and Medzhitov (2007) showed that it is the effect of tumor progression and not initiation that is affected by MyD88 (a signaling adaptor to Toll-like receptors). Thus, examination of parameters at the initiation phase at necrotic doses for liver tumors may not be relevant.

E.3.3.5. *Epigenomic Modification*

There are several examples of chemical exposure to differing carcinogens that have lead to progressive loss of DNA methylation (i.e., DNA hypomethylation) including TCE and its metabolites. The evidence for TCE and its metabolites is specifically discussed in Section E.3.4.2.2, below. Other examples of carcinogens exposures or conditions that have been noted to change DNA methylation are early stages of tumor development include ethionine feeding, phenobarbitol, arsenic, dibromoacetic acid, and stress. However, it has not yet been established whether epigenetic changes induced by carcinogens and found in tumors play a causative role in carcinogenesis or are merely a consequence of the transformed state (Tryndyak et al., 2006).

Pogribny et al. (2007) report the effects of WY-14,643 on global mouse DNA hypomethylation exposed at 1,000 ppm for 1 week, 5 weeks, or 5 months. What is of particular note in this study is that at this exposure level, one commonly used for MOA studies using WY-14,643 to characterize the effects of PPARα agonists as a class, there was significant hepatonecrosis and mortality reported by Woods et al. (2007b). Both wild-type and PPARα -/- null mice were examined. In wild-type mice DNA syntheses was elevated 3-, 13-, and 22-fold of time-matched controls after 1 week, 5 weeks, and 5 months of WY 14,543 treatment. Changes in ploidy were not examined. After 5 weeks of exposure, the ratio of unmethylated CpG cites in whole liver DNA was the same for WY-14,643 treatment and control but by 5 months there was an increase in hypomethylation in WY-14,643 treated wild-type mice. The authors did not report whether foci were present or not which could have affected this result. The similarity in hypomethylation at 5 days and 5 weeks, a time point that also had a small probability of foci development, is suggestive of foci affecting the result at 5 months. For PPAR -/- mice there was increased hypomethylation reported at 1 week and 5 weeks after WY-14,643 treatment that was not statistically significant with so few animals studied. At 5 months the null mice had decreased hypomethylation compared to 1 and 5 weeks. The authors note that, methylation of c-Myc genes was reported to not be affected by long-term dietary treatment with WY-14,643 even though WY-14,643-related hypomethylation of c-Myc gene early after a single dose of WY-14,643 has been observed (Ge et al., 2001a). The authors concluded "thus, alterations in the genome methylation patterns with continuous exposure to nongenotoxic liver carcinogens, such as WY, may not be confined to specific cell proliferation-related genes."

Pogribny et al. (2007) reported Histone H3 and H4 trimethylation status in wild-type and PPAR null mice to show a rapid and sustained loss of histone H3K9 and histone H4K20 trimethylation in wild-type mice fed WY-14,643 from 1 week to 5 months. There was no progressive loss in histone hypomethylation, with the same amount of demethylation occurring at 5 days, 5 weeks, and 5 months in wild-type mice fed WY-14,643. The change from control was ~60% reduction. The control values with time were not reported and all controls were pooled to give one value (n = 15). For PPAR -/- l mice there was a slight decrease with WY-14,643 treatment (~15%) reported. In wild-type mice, WY-14,643 treatment was reported to have no effect on the major histone methyltransferase, Suv39h1, while expression of another (PRDM/Riz1) increased significantly as early as on week of treatment and remained elevated for up to five months. The effect on expression of Suv420h2 (responsible for histone H4K20 trimethylation) was more gradual and the amounts of this protein in livers of mice fed Wy-14m643 were reported to be lower than in control. The authors did not examine these parameters in the null mice so the relationship of these effects to receptor activation cannot be determined. Pogribny et al. (2007) report hypomethylation of retroelements (LTR IAP, LINE1 and LINE2 retrotransposons) following long-term exposure to WY-14,643, which the authors concluded, can have effects on the stability of the genome. Again, these results are for whole liver that may contain foci. Nevertheless, these findings raise questions about other target organs and a more general mechanism for WY-14,643 effects than a receptor mediated one. The lack of effects on c-Myc and the irrelevance of the transient proliferation through it reported here gives more evidence of the irrelevance of a MOA dependent on transient proliferation. The authors noted that studies show that a sustained loss of DNA methylation in liver is an early and indispensable event in hepatocarcinogenesis induced by long-term exposure of both genotoxic and nongenotoxic carcinogens in rodents. Thus, this statement argues against making such a distinction in MOA for "genotoxic" and "nongenotoxic" carcinogens. Finally, the use of a dose which Woods et al. (2007b) demonstrate to have significant hepatonecrosis and mortality, limits the interpretation of these results and their relevance to models of carcinogenesis without concurrent necrosis.

Strain sensitivity to hepatocarcinogenicity has been investigated in terms of short-term changes in methylation. Bombail et al. (2004) reported that a tumor-inducing dose of phenobarbital reduced the overall level of liver DNA methylation in a tumor-sensitive (B6C3F1) mouse strain but that the same dose of phenobarbital did not alter global methylation level in a more tumor-resistant strain (C57BL/6), although the compound increased hepatocyte proliferation as measured by increased DNA synthesis in both strains (Counts et al., 1996). Bombail et al. reported that "In a similar study, Watson and Goodman (2002) used a PCR-based technique to measure DNA methylation changes specifically in GC-rich regions of the mouse genome." Watson and Goodman (2002) found that, that in these areas of the genome, exposure to phenobarbital caused an increase in methylation in dosed animals compared with control animals. Again, the change was more pronounced in tumor-prone C3H/He and B6C3F1 strains than in the less sensitive C57BL/6 strain. They also reported increased DNA synthesis in C57BL/6 mice but decreased global methylation in the B6C3F1 strain after PB administration 1−2 weeks. The lifetime spontaneous tumor rates were reported to be less than 5% in C57BL/6 mice but up to 80% in C3H/He mice. Counts et al. (1996) reported cell proliferation and global hepatic methylation status in relatively liver tumor susceptible B6C3F1 with relatively resistant C57BL6 mice following exposure to PB and/or chlorine/methionine deficient (CMD) diet. Cell proliferation (i.e, DNA synthesis) was reported to be higher in C57BL/6 mice while transient hypomethylation occurred to a greater extent in B6C3F1 mice after phenobarbital treatment. Dual administration of CMD and PB led to enhanced cell proliferation and greater global hypomethylation with similar trends in terms of strain sensitivities in comparison to with either treatment alone (i.e., greater increase in cell proliferation in C57BL/6 and greater levels of hypomethylation in B6C3F1). Thus, the authors concluded that B6C3F1 mice have relatively low capacity to maintain the nascent methylation status of their hepatic DNA. However, on the whole, the control values for methylation for the C57BL/6 mice appear to be slightly higher than the B6C3F1 mice. Thus, claims that the liver tumor sensitive B6C3F1 had more global hypomethylation after a promoting stimulus, which could be related to tumor sensitivity, is tempered by the fact that resistant strain had a higher control baseline of methylation. The baseline level of LI or hepatocyte proliferation also appears to be slightly higher in the C57BL/6 mouse. In addition, the largest strain difference in hypomethylation after a CMD diet was at Week 12 (135% of control for the B6C3F1 strain and 151% of control for the C57BL/6 strain) and this pattern was opposite that for the 1 week time point. Thus, the suggestion by Counts et al. (1996), that the inability to maintain methylation status by the B6C3F1 strain, is also not supported by the longer duration data for CMD diet.

E.3.4. Specific Hypothesis for Mode of Action (MOA) of Trichloroethylene (TCE) Hepatocarcinogenicity in Rodents

E.3.4.1. *PPARα Agonism as the Mode of Action (MOA) for Liver Tumor Induction—The State of the Hypothesis*

PPARα receptor activation has been suggested to be the MOA for TCA liver tumor induction and for TCE liver tumor induction to occur primarily as a result of the presence of its metabolite TCA (NAS, 2006). However, as discussed previously (see Section E.2.1.10), TCE-induced increases in liver weight have been reported in male and female mice that do not have a functional PPARα receptor (Nakajima et al., 2000). The dose-response for TCE-induced liver weight increases differs from that of TCA (see Section E.2.4.2). The phenotype of the tumors induced by TCE have been described to differ from those by TCA and to be more like those occurring spontaneously in mice, those induced by DCA, or those resulting from a combination of exposures to both DCA and TCA (see Section E.2.4.4). As to whether TCA-induced tumors are induced through activation of the PPARα receptor, the tumor phenotype of TCA-induced mouse liver tumors has been reported to have a pattern of H-ras mutation frequency that is opposite that reported for other peroxisome proliferators (see Section E.2.4.4.; Bull et al., 2002; Stanley et al., 1994; Fox et al., 1990; Hegi et al., 1993). While TCE, DCA, and TCA are weak peroxisome proliferators, liver weight induction from exposure to these agents has not correlated with increases in peroxisomal enzyme activity (e.g., PCO activity) or changes in peroxisomal number or volume. However, liver weight induction from subchronic exposures appears to be a more accurate predictor of carcinogenic response for DCA, TCA, and TCE in mice (see Section E.2.4.4). The database for cancer induction in rats is much more limited than that of mice for determination of a carcinogenic response to these chemicals in the liver and the nature of such a response.

The MOA for peroxisome proliferators has been the subject of research and debate for several decades. It has evolved from an "oxidative damage" due to increased peroxisomal activity to a MOA framework example developed by Klaunig et al. (2003) that described causal inferences for hepatocarcinogenesis after a chemical exposure was shown to activate of the PPAR-α receptor with concurrent perturbation of cell proliferation and apoptosis, and selective clonal expansion. Of note although inhibition of apoptosis was proposed as part of the sequellae of PPARα activation, as noted in Section E.2.4.1, no changes in apoptosis in mice exposed to TCE have been reported with the exception of mild enhanced apoptosis at 1,000 mg/kg/d dose but more importantly that for mice the rate of apoptosis decreases as mice age and appear to be lower than that of rats. While DCA exposure has been noted to reduce apoptosis, the significance of DCA-induced reduction in apoptosis from a level that is already inherently low in the mouse, is difficult to apply as the MOA for DCA-induced liver cancer.

Klaunig et al. based causal inferences on the attenuation of these events in PPAR-α-null mice in response to the prototypical agonist WY-14,643 with a number of intermediary events considered to be associative (*e.g.*, expression of peroxisomal and nonperoxisome genes, peroxisome proliferation, inhibition of gap junction intracellular communication, hepatocyte oxidative stress as well as Kupffer cell-mediated events). The data set for DEHP was prominently featured as an example of "PPAR-α induced hepatocarcinogenesis." For DEHP PPAR-α activation was described as the initial key event with evidence lacking for a direct effect but supported primarily supported by evidence from PPAR-α-knockout mice treated with WY-14,643. Klaunig et al. concluded that "…all the effects observed are due only to the activation of this receptor and the downstream events resulting from this activation and that no other modes of action are operant"

Although that PPARα receptor activation is the sole MOA for DEHP has been cited by several reports (including IARC, 2000), several articles have questioned the adequacy of this proposed MOA (Melnick, 2001, 2002, 2003; Melnick et al., 2007; FIFRA SAP, 2004; Caldwell and Keshava, 2006; Caldwell et al., 2008b; Keshava and Caldwell, 2006; and Keshava et al., 2007; Guyton et al. 2009). New information is now available that also questions several of the assumptions inherent in the proposed MOA by Klaunig et al. and the dismissal of PPARα agonists as posing a health risk to humans. Specific questions have been raised about the use of WY-14,643 as a prototype for PPARα (especially at necrogenic doses) and use of the PPARα -/- null mouse in abbreviated bioassays to determine carcinogenic hazard.

E.3.4.1.1. ***Heterogeneity of PPARα agonist effects and inadequacy of WY-14,643 paradigm as prototype for class.*** Inferences regarding the carcinogenic risk posed to humans by PPARα agonists have been based on limited epidemiology studies in humans that were not designed to detect such effects. However, as noted by Nissen et al. (2007) the PPARα receptor is pleiotropic, highly conserved, has "cross talk" with a number of other nuclear receptors, and plays a role in several disease states. "The fibrate class of drugs, which are PPARα agonists intended to treat dyslipidemia and hypercholesterolemia, have recently been associated with a number of serious side effects." While these reports of clinical side effects are for acute or subchronic conditions and do not (and would not be expected to) be able to detect liver cancer from fibrate treatment, they clearly demonstrate that compounds activating the PPAR receptors may produce a spectrum of effects in humans and the difficulty in studying and predicting the effects from PPAR agonism. Graham et al. (2004) recently reported significantly increased incidence of hospitalized rhabdomyolysis in patients treated with fibrates both alone and in combination with statins. Even though pharmaceutical companies have spent a great deal of effort to develop agonists which are selective for desired effects, the pleiotropic nature of the receptor continues to be an obstacle.

Also, fibrates, WY-14,643 and other PPARα agonists are pan agonists for other PPARs. Shearer and Hoekstra (2003) note that fibrates, including Fenofibrate, Clofibrate, Bezafibrate, Ciprofibrate, Gemfibrozil, and Beclofibrate are all drugs that were discovered prior to the cloning of PPARα and without knowledge of their mechanism of action but with optimization of lipid lowering activity carried out by administration of candidates to rodents. They report that many PPARα ligands, including most of the common fibrate ligands, show only modest selectivity over the other subtypes with, for example, fenofibric acid and WY-14,643 showing <10-fold selectivity for activation of human PPARα compared to PPARγ and/or PPARδ. In human receptor transactivation assays they report:

Human receptor transactivation assays of median effective concentration (EC_{50}):

WY-14,643 = 5.0 μm for PPARα, 60 μm for PPAR γ, 35 μm for PPARδ.

Clofibrate = 55 μm for PPARα, ~500 μm for PPAR γ, inactive at 100 μm for PPARδ

Fenofibrate = 30 μm for PPARα, 300 μm for PPAR γ, inactive at 100 μm for PPARδ
Bezafibrate = 50 μm for PPARα, 60 μm for PPAR γ, 20 μm for PPARδ.

Murine receptor transactivation assay of EC_{50}:

WY = 0.63 μm for PPARα, 32 μm for PPAR γ, inactive at 100 μm for PPARδ
Clofibrate = 50 μm for PPARα, ~500 μm for PPAR γ, inactive at 100 μm for PPARδ
Fenofibrate = 18 μm for PPARα, 250 μm for PPAR γ, inactive at 100 μm for PPARδ
Bezafibrate = 90 μm for PPARα, 55 μm for PPAR γ, 110 μm for PPARδ.

Thus, these data show the relative effective concentrations and "potency for PPAR activity" of various agonists in humans and rodents, rodent and human responses may vary depending on agonist, agonists vary in what they activate between the differing receptors, and that there is a great deal of transactivation of these drugs.

For fibrates specifically, a study by Nissen et al. (2007) reports that in current practice, 2 fibrates, Gemfibrozil and Fenobibrate, are still widely used to treat a constellation of lipid abnormalities known as atherogenic dyslipidemia and note that currently available fibrates are weak ligands for the PPARα receptor and may interact with other PPAR systems. They note that the pharmaceutical industry has sought to develop new, more potent and selective agents within this class but, most importantly, that none of the novel PPARα agonists has achieved regulatory approval and that according to a former safety officer in the U.S. Food and Drug Administration (El-Hage, 2007) that more than 50 PPAR modulating agents have been discontinued due to various types of toxicity (e.g., elevations in serum creatinine, rhabdomylosis, "multi-species, multi-site increases in tumor with no safety margin for clinical exposures," and adverse cardiovascular outcomes) but without scientific publications describing the reasons for termination of the development programs. Nissen et al. report differences in effect between a more highly selective and potent PPARα agonist and the less potent and specific one in humans. They note

> a recent large study of Fenofibrate in patients with diabetes showed no significant reduction in morbidity but a trend toward increased all-cause mortality (Keech et al. 2005, 2006). Whether this potential increase in mortality is derived from compound specific toxicity of Fenofibrate or is an adverse effect of PPARα activation remains uncertain."

In addition to the lack of publication of effects from PPAR agonists in human trials in which toxicity can be examined as noted by Nissen et al., the Keech study

> is illustrative of the problem in trying to ascertain liver effects from fibrate treatment in humans as the focus of the outcomes was coronary events in a study of 5 years duration in a older diabetic population. As stated above, the challenges the pharmaceutical industry and the risk assessor face in determining the effects of PPAR agonists is "that these compounds and drugs modulate the activity of a large number of genes, some of which produce unknown effects."

Nissen et al. further note that

> Accordingly, the beneficial effects of PPAR activation appear to be associated with a variety of untoward effects which may include, oncogenesis, renal dysfunction, rhabdomylosis, and cardiovascular toxicity. Recently, the FDA began requiring 2-year preclinical oncogenicity studies for all PPAR-modulating agents prior to exposure of patients for durations of longer than 6 months (El-Hage, 2007).

Guyton et al. (2009) further explore the status of the PPARα epidemiological database and describe its inability to discern a cancer hazard from the available data. Thus, while existing evidence for liver cancer in humans is null rather than negative, there remains a concern for oncogenicity and many obstacles for determining such effects through human study. The heterogeneity in response to PPARα agonists and the heterogeneity of effects they cause (Keshava and Caldwell, 2006) are evident from these reports.

Many studies have used the effects of WY-14,643 at a very high dose and extrapolated those findings to PPARα agonists as a class. However, this diverse group of chemicals have varying potencies and effects for the "key events" described by Klaunig et al. (2003) (Keshava and Caldwell, 2006). The standard paradigm used with WY-14,643 to induced liver tumors in all mice exposed to 1 year (an abbreviated bioassay), uses a large dose that has also has been reported to produced liver necrosis, which can have an effect of cell proliferation and gene expression patterns, and to also induce premature mortality (Woods et al., 2007b). As stated above, WY-14,643 also has a short peak of DNA synthesis that peaks after a few days of exposure, recedes, and then unlike most PPARα agonists studied (e.g., Clofibrate, clofibric acid, Nafenopin, Ciprofibrate, DEHP, DCA, TCA and LY-171883) has a sustained proliferation at the doses studied (Tanaka et al., 1992; Barrass et al., 1993; Marsman et al., 1992; Eacho et al., 1991; Lake et al., 1993; Yeldani et al., 1989; David et al., 1999; Marsman et al., 1988; Carter et al., 1995; Sanchez and Bull, 1990). Clofibrate has been shown to have a decrease in proliferation gene expression shortly after its peak (see Section E.3.2.2). As shown in above for WY-14,643, hepatocellular increases in DNA synthesis did not appear to have a dose-response (see Section E.3.4.2), only WY-14,643 had a sustained elevation of Nf-κB (gem and dibutyl phthalate did not) (see Section E.3.4.3.3), and the effects on DNA methylation occurred at 5 months and not earlier time points (when Foci were probably present) and effects of histone trimethylation were observed to be the same from 1 weeks to 5 months (see Section E.3.4.5). Such effects on the epigenome suggest other effects of WY-14,643, other than receptor activation, are not specific to just WY-14,643 and are found in a number of conditions leading to cancer and in tumor progression (see Sections E.3.2.1 and E.3.2.7.).

In their study of PPARα-independent short-term production of reactive oxygen species from induced by large concentrations of WY-14,643 and DEHP in the diet, Woods et al. (2007c) examined short-term exposures to (0.6% w/w DEHP or 0.05% or 500 pm WY-14,643 for 3 days, 1 weeks or 3 weeks) and reported that WY-14,643 induced a dramatic increase in bile flow that was not observed from DEHP exposure. By 1 week of exposure there was a 5% increase in bile flow for DEHP treatment but a 240% increase in bile flow for WY-14,643 treatment. By 3 weeks the difference in bile volume between treated and control was 12% for DEHP and 1,100% for WY-14,643 treated animals. In this study oxygen radical formation, as measured by spin trapping in the bile, was reported to be decreased after 3 days of treatment after DEHP and WY-14,643 treatment. However, the large changes in bile flow by WY-14,643 treatment limit the interpretation of these data along with a small number of animals examined in this study (e.g., 6 control and DEHP animals and 3 animals exposed to WY-14,643 at 3 days), a 30% variation in percent liver/body weight ratios between control groups, and the insensitivity of the technique. In an earlier study oxidative stress appears to be correlated with neither cell proliferation nor carcinogenic potency (Woods et al., 2006). Woods et al. (2006) reported WY-14,643Y or DEHP to induce an increase in free radicals at 2 hrs, a decrease at 3 days then an increase at 3 weeks for both. However, radical formation did not correlate with the proliferative response, as DEHP fails to produce a sustained induction of proliferative response in rodent liver but WY-14,643 does, and both WY-14,643 and DEHP gave a similar pattern of radical formation that did not vary much from controls which is in contrast to their carcinogenic potency.

Although assumed to be a reflection of cell proliferation in many studies of WY-14,643 and by Klaunig et al. (2003), DNA synthesis recorded using the standard exposure paradigm for WY-14,643, can also be a reflection of hepatocyte, nonparenchymal cell or inflammatory cell mitogenesis (in the case of necrosis induced inflammation), from changes in hepatocyte ploidy, or a combination of all. Other peroxisome proliferators have been shown to have a decrease in proliferation gene expression shortly after their peaks (e.g., Clofibrate, see Section E.3.2.2) and both Methylclofenapate and Nafenopin have been shown to increase cell ploidy with Nafenopin having the majority of its DNA synthesis a reflection of increased ploidy with only a small percentage as increases in cell number (see Section E.3.4.1). Several authors have also noted increases in ploidy for WY-14,643 (see Section E.3.4.1).

The Tg.AC genetically modified mouse was used to study 14 chemicals administered by the topical and oral (gavage and/or diet) routes by Eastin et al. (2001). Clofibrate was considered clearly positive in the topical studies but not WY-14,643 regardless of route of administration. Based on the observed responses, it was concluded by the workgroup (Assay Working Groups) that the Tg.AC model was not overly sensitive and possesses utility as an adjunct to the battery of toxicity studies used to establish human carcinogenic risk. The difference in result between Clofibrate and WY-14,643 is indicative of a different MOA for the two compounds.

Similarly, at large exposure concentrations Boerrigter (2004) investigated the response of male and female lacZ-plasmid transgenic mice treated at 4 months of age with 6 doses of 2,333 mg/kg DEHP, 200 mg/kg WY-14,643 or 90 mg/kg Clofibrate over a two week period. Mutation frequencies were assayed at 21 days following the last exposure. DEHP and WY-14,643 were shown to significantly elevate the mutant frequency in both male and female liver DNA while Clofibrate, at the dose level studied, was apparently nonmutagenic in male and female liver (i.e., six-dose exposure to DEHP or WY-14,643 over a two week period significantly increased the mutant frequency in liver of both female and male mice by approximately 40%). The author noted that

> the laxZ plasmid-based transgenic mouse mutation assay is somewhat unique among other commercially available models (e.g. mutamouse and big blue), by virtue of its ability to accurately quantify both point mutations and large deletions including those which originate in the lacZ plasmid catamer and extend into the 3' flanking genomic region. It should be noted that to date there is no single, agreed upon protocol for conducting mutagenicity assays with transgenic rodents although several aspects have been upon by the Transgenic Mutation Assays workgroup of the International Workshop on Genotoxicity Procedures.

For several chemicals both rats and mice demonstrate evidence of receptor activation through peroxisome proliferation and peroxisome-related gene expression but only one develops cancer. The herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), is a striking example of the problems that would be associated with only using evidence of PPARα receptor activation to make conclusions about MOA of liver tumors. 2,4-D is structurally similar to the PPARα agonist Clofibrate and has been shown at similar concentrations to increase peroxisome number and size, increase hepatic carnitine acetyltransferase activity and catalase, and decrease serum triglycerides and cholesterol in rats (Vainio et al., 1983). Peroxisome number was also increased in Chinese hamsters to a similar level as with Clofibrate at the same exposure concentration after

9 days of exposure to 2,4-D (Vainio et al., 1982). In mice, Lundgren et al. (1987) report that 2,4-D exposure statistically increased the liver-somatic index over controls after a few days exposure and increased mitochondrial protein, microsomal protein, carnitine acetyltransferase, PCO activity, cytochrome oxidase, cytosolic epoxide hydrolase, microsomal epoxide hydrolase, microsomal P450 content, and hepatic cytosolic epoxide hydrolase in mouse liver. Thus, 2,4-D activates the PPARα receptor, with associated changes in peroxisome-related gene expression, in multiple species and at similar doses to Clofibrate. However, Charles et al. (1996) and Charles and Leeming (1998) report that in several 2-year studies that there were no 2,4-D-induced increases in liver tumors in F344 rats, CD-1 rats, B6C3F1 mice and CD-1 mice. Another example, is provided by Gemfibrozil, known as (5-2[2,5-dimethylphenoxy] 2-2-dimethylpentanoic acid) and [2,2-dimethyl-5-(2,5-xylyoxy) valeric acid], a therapeutic agent that activates the PPARα receptor and is a peroxisome proliferator, but is carcinogenic only in male rats but not female rats, nor in either gender of mouse (Contrera et al., 1997). Gemfibrozil causes tumors in pancreas, liver, adrenal, and testes of male rats and causes increases in absolute and relative liver weights in both rats and mice (Fitzgerald et al., 1981). Gemfibrozil, is a highly effective lipid and cholesterol lowering drugs in humans and in mice (Olivier et al., 1988). However, although Gemfibrozil activates the PPARα receptor and induces peroxisome proliferation in mice, it does not induce liver tumors in that species. In the long-term study of Bezafibrate, Hays et al. (2005) note that the role of this receptor in hepatocarcinogenesis has only been examined using one relatively specific PPARα agonist (WY-14,643) and report that Bezafibrate can induce the expression of a number of PPARα target genes (acyl CoA oxidase and CYP4a) and increased liver weight in PPARα knockout mice that is not dependent on activation of PPARβ or PPARγ. As noted by Boerrigter (2004),

> In contrast to DEHP and WY-14,643, Clofibrate produced hepatocellular carcinomas in rats only while no increase in the incidence of tumors was reported in mice (Gold and Zeiger 1997). However, Clofibrate induces peroxisome proliferation in both rats and mice (Lundgren and DePierre 1989) but only produced hepatocellular carcinomas in rats (Gold and Zeiger, 1997).

Melnick et al. (1996) noted that similar levels of peroxisomal induction were observed in rats exposed to DEHP and di(2-ethylhexyl) adipate (DEHA) at doses comparable to those used in the bioassays of these chemicals. However, DEHP but not DEHA gave a positive liver tumor response in 2-year studies in rats. In an evaluation of the carcinogenicity of tetrachloroethylene, an expert panel of the International Agency for Research on Cancer concluded that the weak induction of peroxisome proliferation by this chemical in mice was not sufficient to explain the high incidence of liver tumors observed in an inhalation bioassay.

In adult animals, apoptosis acts as a safeguard to prevent cells with damaged DNA from progressing to tumor, but like cell proliferation, alterations in apoptosis are common to many MOAs. In addition, only short-term data are available on changes in apoptosis due to PPARα agonists, and long-term changes have not been investigated (Rusyn et al., 2006). For example, although a decrease in apoptosis has also suggested to be an important additional molecular event that may affect the number of cells in rodent liver following exposure to the peroxisome proliferator DEHP, apoptosis rates have not investigated past 4 days of exposure and thus, the time-course of this event is uncertain. The antiapoptotic effects of PPAR agonists appear to be also dependent on nonparenchymal cells (i.e., Kupffer cells) which do not express PPARα and could be a transient event (Rusyn et al., 2006). Morimura et al. (2006) report evidence for exposure to WY-14,643 that does not support a role for PPARα–mediated apoptosis in tumor formation (see Section E.3.5.1.3, below) as well as appearing to be specific to WY-14,643 (see Section E.3.4.3.3).

The lack of a causal relationship of transient DNA synthesis increases and hepatocarcinogenesis has been raised by many (Caldwell et al., 2008b) and is discussed in Section E.3.4.2 as well as the changes in ploidy (see Section E.3.4.1). In regard to gene expression profiles, many studies have focused on gene profiles during the early transient proliferative phase or have identified genes primarily associated with peroxisome proliferation as "characteristic" or relevant to those associated with tumor induction. Several have focused on the number of genes whose expression "goes up" or "goes down" from a small number of animals. Caldwell and Keshava (2006) presented information on WY-14,643, dibutyl phthalate, Gemfibrozil and DEHP, and noted inconsistent results between PPARα agonists, paradoxes between mRNA and protein expression, strain, gender, and species differences in response to the same chemical, and time-dependent differences in response for several enzymes and glutathione.

E.3.4.1.2. ***New information on causality and sufficiency for PPARα receptor activation.*** In its review of the U.S. EPA's draft risk assessment of perfluorooctanoic acid (PFOA), the Science Advisory Panel (FIFRA SAP, 2004) expressed concerns about whether PPARα agonism constitutes the sole MOA for PFOA effects in the liver and the relevance to exposed fetuses, infants, and children. In part based on uncertainties regarding the Klaunig et al. (2003) proposed MOA, they concluded that the tumors induced by PFOA were relevant to human risk assessment. The hypothesis that activation of the PPARα receptor is the sole mode of action hepatocarcinogenesis induced by DEHP and many other chemicals is further called into question by recent studies. In the case of DEHP, Klaunig et al. (2003) assumed that WY-14,643 and DEHP would operate through the same key events and that long-term bioassays of DEHP in PPARα -/- knockout mice would be negative and hence demonstrate the need for receptor activation for hepatocarcinogenesis from DEHP.

The fallacy of these assumptions is illustrated by the recent report of the first 2-year bioassay of DEHP in PPARα -/- knockout mice (Sv/129 background strain) that reported DEHP-induced hepatocarcinogenesis (Ito et al., 2007). Further discussion is provided by Guyton et al. (2009). Similar to other studies, the PPAR -/- mice had slightly increased liver weights in comparison to controls and treated wild-type mice (~12% increase over controls). In fact statistical analysis of the incidence data show that adenomas were significantly increased in PPARα -/- mice compared with wild-type mice exposed to 500 ppm DEHP and that a significant dose-response trend for adenomas and adenomas plus carcinomas was observed in PPARα -/- mice (Figure E-5). Overall, the cancer incidences were consistent with a previous study of DEHP (David et al., 1999) in B6C3F1 mice at the same doses for nearly the same exposure duration. A strength of this study is that it was conducted at much lower more environmentally relevant doses that did not significantly increase liver enzymes as indications of toxicity. As noted by Kamija et al. (2007), DEHP was reported also to induce glomerularnephritis more often in PPARα-null mice because of the absence of PPARα-dependent anti-inflammatory effect of antagonizing the oxidative stress and NF-κB pathway (Kamijo et al., 2007). Thus, these data support that hypothesis that there is no difference in liver tumor incidences between PPARα -/- mice and wild-type mice in a standard nonabbreviated exposure bioassay that does not exceed the maximal tolerated doses and that DEHP can induce hepatotoxicity as well as other effects independent of action of the PPARα receptor.

The study of Yang et al. (2007a) informs as to the sufficiency of PPARα receptor activation and subsequent molecular event for hepatocarcinogenesis in mice. The study used a VP16PPARα transgene under control of the liver-enriched activator protein (LAP) promoter to activate constitutively the PPARα receptor in mouse hepatocytes. LAP-VP16PPARα transgenic mice showed a number of effects associated with PPARα receptor activation including decreased serum triglycerides and free fatty acids, peroxisome proliferation, enhanced hepatocyte DNA synthesis and induction of cell-cycle genes and those described as “PPARα targets” to comparable levels reported for WY-14,643 exposure. Hepatocyte proliferation, as determined by the labeling index of hepatocyte nuclei, was increased after 2 weeks of WY-14,643 treatment over controls (20.5 vs. 1.6% in control livers) with the LAP-VP16PPARα mice giving a similar results (20.8 vs. 1.0% in control livers). The authors noted that transgenic mice did not appear to have positive labeling of nonparenchymal cell nuclei that were present in the WY-14,643 treated animals. The transferase-mediated dUTP nick end-labeling assay results were reported to show that there was no difference in apoptosis in wild-type mice treated with WY-14,643, the transgenic mice, or controls. In a small number of animals, microsomal genes (CYP4A), peroxisomal (Acox, BIEN—the bifunctional enzyme) and mitochondrial fatty oxidation genes (LCAD—long chain acyl CoA dehydrogenase and VLCAD—very long chain acyl CoA dehydrogenase) were expressed in the transgenic mice with WY-14,643 also increasing expression of these genes in wild-type mice but with less lipoprotein lipase (LPL) than the transgenic mice. Hepatic CoA oxidation, were increased to a similar level in wild-type mice treated with WY-14,643 and the transgenic mice (*n* = 3−4) and were statistically different than controls. LAP- VP16PPARα transgenic mice (8 weeks of age) exhibited hepatomegaly (~50 increase percent body/liver weight over controls), and an accumulation of lipid due to triglycerides but not cholesterol. However, compared to wild-type mice exposed to WY-14,643 for two weeks, the extent of hepatomegaly was reduced (i.e., percent liver/body weight increase of ~2.5-fold with WY-14,643 treatment), no hepatocellular hypertrophy or eosinophilic cytoplasms and no evidence of nonparenchymal cell proliferation were observed in the LAP-VP16PPARα transgenic mice.

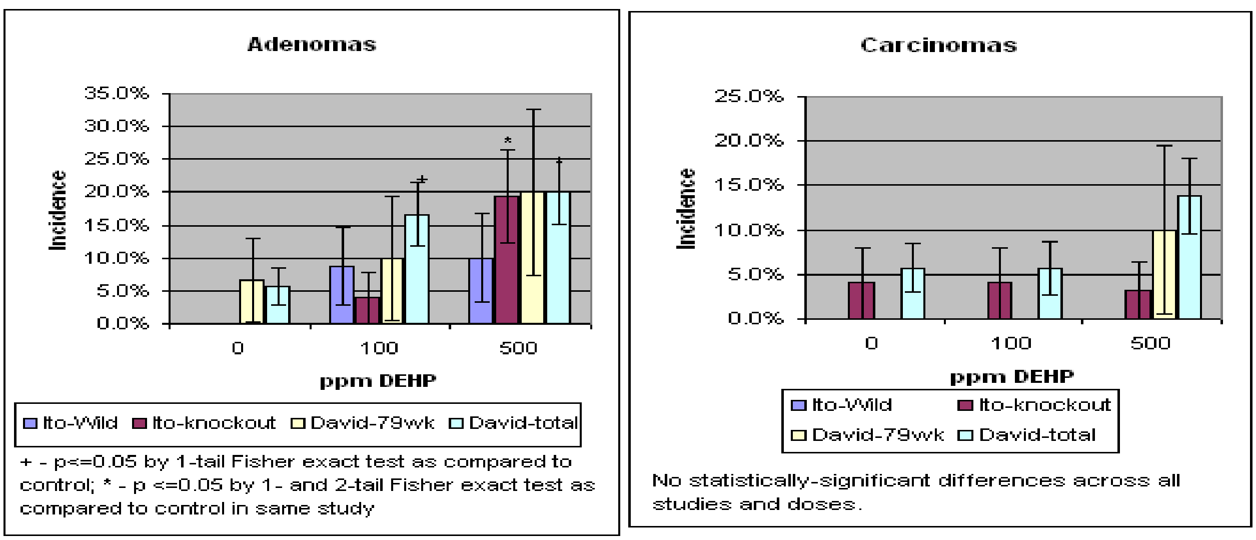


Figure E-5. Comparison of Ito et al. and David et al. data for DEHP tumor induction from Guyton et al. (2009).

At ~1 year of age, Yang et al. (2007a) reported there to be no evidence of preneoplastic lesions or hepatocellular neoplasia in LAP- VP16PPARα transgenic mice, in contrast to results after 11 months of exposure to WY-14,643 in wild-type mice. Microscopic examination of liver sections were consistent with the gross findings, as hepatocellular carcinomas and hepatic lesions were observed in the long-term WY-14,643 treated wild-type mice, but not in >20 LAP-VP16PPARα mice at the age of over 1 year in the absence of dox. There was no quantitative information on tumors given nor of foci development in the WY-14,643 mice. As noted by Yang et al. (2007a), PPARα activation only in mouse hepatocytes is sufficient to induce peroxisome proliferation and increased DNA synthesis but not to induce liver tumors. Thus, "hepatocyte proliferation" indentified by Klaunig et al. (2003) as a "causal event" in their PPARα MOA is not sufficient to induce hepatocarcinogenesis. These data not only call into question the adequacy of the MOA hypothesis proposed by Klaunig et al. (2003) but suggest multiple mechanisms and also multiple cell types may be involved in hepatocarcinogenicity caused by chemicals that are also PPARα agonists.

E.3.4.1.3. ***Use of the PPAR -/- knockout and humanized mouse.*** Great importance has been attached to the results reported for PPARα -/- mice and their humanized counterpart with respect to inferences regarding the MOA or peroxisome proliferators and whether short-term chemical exposures or abbreviated bioassays conducted with these mice can show that a PPARα MOA is involved. Consequently, the use of these models warrants scrutiny. Compared to untreated wild-type mice, liver weights in knockout mice or humanized mice have been reported to be elevated (Voss et al., 2006; Laughter et al., 2004; Morimura et al., 2006) and within 10% of each other (Peters et al., 1997). In order to be able to assign affects to a test chemical tested in knockout mice, a better characterization is needed of the baseline differences between PPARα -/- knockout and wild-type mice. This is particularly important for examining weak agonists because the changes they induce may be small and need to be confidently distinguished from differences due to the loss of the receptor alone. As shown by the Ito et al. (2007) study and as noted by Maronpot et al. (2004), there is a need for lifetime studies to characterize background or spontaneous tumor patterns and life spans (including those of the background strain). While the original work by Lee et al. (1995) describes "the mice homozygous for the mutation were viable, healthy, and fertile and appeared normal," the authors did not describe the survival curves for this model nor their background tumor rate. In fact, further work has shown that they carry a background of chronic conditions, including: (1) chronic diseases such as obesity and steatosis (Akiyama et al., 2001; Costet et al., 1998); (2) altered hepatic of hepatocellular structure and function, such as vacuolated hepatocytes (Voss et al., 2006; Anderson et al., 2004), also seen in "humanized" mice (Cheung et al., 2004); and (3) altered lipid metabolism, including reduced glycogen stores, blunted hepatic and cardiac fatty acid oxidation enzyme system response to fasting, elevated plasma free fatty acids, fatty liver (steatosis), impaired gluconeogenesis, and significant hepatic insulin resistance (Lewitt el al., 2001). Howroyd et al. (2004) reported decreased longevity and enhancement of age-dependent lesions in PPARα -/- mice.

These baseline differences from wild-type mice may render them more susceptible to toxic responses or shorten their lifespans with chemical exposure. For example, after administration of 250 microliters CCl_4/kg, all male and 40% of female PPARα knockout mice were dead or moribund after 2 days of treatment, whereas 25% of male wild-type mice and none of the female wild-type mice exhibited outward signs of toxicity (Anderson et al., 2004). Hays et al. (2005) reported that 100% of PPARα knockout have cholestasis after 1 year of Bezafibrate treatment with higher bile acid concentration than wild-type mice. Lewitt et al. (2001) noted that male knockout mice have more marked accumulation of hepatic fat, hypercholesterolemia and to be particularly sensitive to fasting with some dying if fasted for more than 24 hours. Sexual dimorphism but especially increased susceptibility of the male mouse has been reported for knockout mice with pure Sv/129 backgrounds (Lewitt et al., 2001; Anderson et al., 2004) as well as those with a suggested C57BL/6N background (Djouadi et al., 1998, Costet et al., 1998). Akiyama et al. (2001) showed an apparent greater sexual dimorphism in mice with a pure Sv/129 background than C57BL/6N in regard to weight gain from 2 to 9 months but not in changes in body weight or liver weight between wild-type and knockout animals. Adipose tissue, serum triglycerides and cholesterol were altered in the knockout animals. Given that the experiment was only carried out for 9 months, changes in body fat, liver weight and lipid levels may be greater as the animals get older and steatosis is more prevalent. The dramatic effect on survival as well as gender difference by the increased expression of lipoprotein lipase in the PPARα knockout mouse with further genetic modification is demonstrated by Nohammer et al. (2003) who reported 50% mortality in 6 months and 100% mortality within 11 months of age while females survived. These differences could affect the results of tumor induction for PPARα agonists with less potency than WY-14,643 that do not produce tumors so rapidly. In addition, these studies suggest the need for careful consideration of the effects of use of different background strains for the knockout and the need for careful characterization of the background responses of the mouse model and the effects of the use of different background strains for the knockout. Morimura et al. (2006) reported that, using the B6 background strain, there were only foci at time periods but knockouts with the SV129 background had multiple tumors after WY-14,643 treatment.

PPARα knockout mice have also been used to examine the dependence of PPARα on changes in cell signaling, protein production, or liver weight. However, to be useful, the changes incurred just by loss of the PPARα should also be well described. Reported differenced between PPARα-knockout and wild-type mice can impact the sensitivity and specificity of these markers of for the hypothesized MOA. In regards to altered cell signaling, Wheeler et al. (2003) note that in normal cells $p21^{waf}$ and $p27^{kip1}$ inhibit the Cdk/cyclin complexes responsible for cell cycle progression through G1/S transition. While these cellular signaling molecules are down-regulated in response to partial hepatectomy in normal mice, they remain elevated in PPARα knockout mice along with decreased DNA synthesis. Fumonisins are hepatocarcinogens that have been associated changes in apoptosis and tissue generation, and increased acyl-CoA oxidase and CYP4A (markers of PPARα activation) (Martinez-Larranaga et al., 1996). Voss et al. (2006) report that the average number of hepatic apoptotic foci per mouse induced by Fumonisins were 3-fold higher and liver mitotic figures counts were 2-fold lower in PPARα knockout in comparison to wild-type mice, thus, illustrating a difference in proliferative response in the mice. PPARα-null mice have been reported to have increased apoptosis and decreased mitosis with fumonisin treatment. Voss et al. (2006) also report several differences in gene expression in wild-type and PPARα knockout mice that ranged from 0.3 to 483% of the activity of wild-type mice. The complex expression patterns of gene expression and determination of their mechanistic implications in regard to hepatotoxicity and carcinogenicity are difficult. Certainly the large number of genes whose expression is affected by WY-14,643 (1,012 genes as cited by Voss et al., 2006) illustrates such complexity. Voss et al. (2006) conclude that studies should consider dose- and time course-related effect as well as species and strain-related differences in the expression of gene products.

The "humanized" PPARα mouse has a human copy of PPARα inserted into a PPARα knockout mouse. It is inserted in a tetracycline response system so that in the absence of DOX only human PPARα is transcribed in humanized mouse liver and not in other tissues. A rigorous examination of newly emerging studies regarding the "humanized" mouse is warranted. There are two papers that have been published using the humanized PPARα mouse (Cheung et al., 2004; Morimura et al., 2006). Many of the issues described above for PPARα -/- mice are of concern for the humanized knockout mouse. In addition, the placement of the humanized PPAR gene is a potential confounding factor, as discussed by Morimura et al. (2006):

> It also cannot be ruled out that the hPPARα mice are resistant to the hepatotoxic effects of peroxisome proliferators due to the site of expression of the human receptor. The cDNA was placed under control of the tetracycline regulatory system and the liver-specific Cebp/B promoter that is preferentially expressed in hepatocytes.

In the Cheung et al. (2004) report, the humanized mouse was fed WY-14,643 for 2 or 8 weeks (age not given for the mice). WY-14,643 and Fenobrate were reported to decrease serum total triglyceride levels in wild and humanized mice to about the level seen in PPARα -/- mice (which were already suppressed without treatment). Hepatomegaly and increase in hepatocyte size were observed in the PPARα -humanized mice fed WY-14,643 for 2 weeks but less than that of wild mice. By contrast, Morimura et al., (2006) state that the humanized mice did not exhibit hepatomegaly after treatment with WY-14,643. Cheung et al (2004) present figures that show increased vacuolization of hepatocytes in a control humanized mouse in comparison to wild-type mice. Vacuolization increased with WY-14,643 treatment in the humanized mouse. Therefore, there was a background level of liver dysfunction in these mice even with humanized PPARα. Vacuolization is consistent with fatty liver observed in the nonhumanized PPARα -/- mouse. The authors reported that the humanized mouse did not have increased #s of peroxisomes after WY treatment. However, they present a figure for genes encoding peroxisomal, mitochondrial, and microsomal fatty acid oxidation enzymes that shows they were still markedly increased in PPARα -humanized mice following 8 weeks of exposure to WY-14,643. Therefore, there is a paradox in these reported results.

Morimura et al. (2006) provided a useful example to illustrate the many issues associated with interpreting studies with genetically-altered animals. While this study is suggestive of a difference in susceptibility to tumor induction between wild-type and PPARα humanized mice, a conclusion that human PPARα is refractory to liver tumor induction is not sufficiently supported by this study. This study had uneven durations of exposure and follow-up and reported substantial toxicity or mortality that limit the interpretation of the observed tumor rates. For example, the 6 week-old male "humanized" mice had a 44-week experimental period but for wild-type mice that period was 38 weeks. In addition, for humanized mice, 10 mice were treated with 0.1% WY-14,643 with 20 controls, but for wild-type mice, 9 mice were given 0.1% WY with 10 controls. Furthermore, wild-type, WY-14,643-treated animals had suppressed growth and only a 50% survival to 38 weeks, so an effective LD_{50} has been used for this length of exposure. Specifically, of the 10 wild-type WY-14,643 treated mice, 3 died of toxicity and 2 were killed due to morbidity and their tissues examined. Humanized mice had similar growth for animals treated with WY-14,643 or controls with only one mouse killed because of morbidity. Therefore, the reported results, including tumor numbers, are for a mixture of different exposure durations and ages of animals. In addition the results of the study were reported for only on exposure level.

Furthermore, it is interesting that while control humanized mice had no adenomas, WY-14,643 treated humanized mice had one. Morimura et al. (2006) noted that this adenoma had a morphology "similar to spontaneous mouse liver tumor with basophilic and clear hepatocytes," whereas the tumors in wild-type mice treated with WY-14,643 were more diffusely basophilic. If the humanized animals were allowed to live their natural lifespan, this raises the possibility that WY-14,643 may induce tumors that are similar to other carcinogens rather than those that have been described as "characteristic" of peroxisome proliferators (see Section E.3.5.1.5) when human PPARα is present. Therefore, the humanized PPARα rather than mouse PPARα may have an association with a tumor phenotype characteristic of other MOAs but this study need to be carried out for a longer period of exposure and with more animals to make that determination. The baseline tumor response of PPARα humanized mice needs to be characterized as well as tumors exposure to WY-14,643 or other carcinogens acting through differing MOAs. The numbers of foci were not reported, but "altered foci" were detected in one humanized mouse with WY-14,643 treatment and one without treatment. The phenotypes of the foci were not given by the authors.

As discussed above, changes in liver weights have been associated with susceptibility to liver tumor induction and the issues regarding baseline differences in PPARα -/- mice are equally relevant for PPARα humanized mice. Morimura et al. (2006) reported that absolute liver weight for control humanized mice at 44 weeks was 1.57 g (n = 10). The absolute liver weight for wild control mice was 1.1 g (n = 9) at 38 weeks. The final body weights differed by 14% but liver weights differed by 30%. Therefore, even though comparing different aged mice, the control humanized mice had greater liver size than the wild-type control mice on an absolute and relative basis. This is consistent with humanized knockout mice having greater sized livers and a baseline of hepatomegaly. With treatment, Morimura et al. (2006) report that PPARα humanized mice treated with WY-14,643 had greater absolute and relative liver weights than controls but less elevations than wild-type treated animals. However, because half of the wild-type animals died, it is difficult to discern if liver weights were reported for moribund animals sacrificed as well as animals that survived to 38 weeks for wild-type mice treated with WY-14,643. However, it appears that moribund animals were included that were sacrificed early for treated groups and that values from the animal killed at 27 weeks were added in with those surviving till 45 weeks in the PPARα humanized mice treated with WY-14,643 group.

With respect to the gene expression results reported by Morimura et al. (2006), it is important to note that they are for liver homogenates with a significant portion of the nuclei from nonparenchymal cell of the liver (e.g., Kupffer and stellate cells). Thus, the results represent changes resulting from a mixture of cell types and from differing zones of the liver lobule, with potentially different gene changes merged together. Livers without macroscopic nodules were used for western blot and but could have contained small foci in the homogenate as well. The gene expression results were also reported for an exposure level of WY-14,643 that is an LD_{50} in wild-type mice and could reflect toxicity responses rather than carcinogenic ones. The samples were also obtained at the end of the experiment (with a mix of durations of exposure) and may not reflect key events in the causation of the cancer but events that are downstream.

These limitations notwithstanding, it is interesting that expression of p53 gene was reported by Morimura et al. (2006) to be increased in PPARα humanized mice treated with WY-14,643 compared to all other groups. Furthermore, of the cell cycle genes that were tested, (i.e., *CD-1*, *Cyclin-dependent Kinases 1* and *4*, and *c-myc*) there was a slightly greater level of *c-myc* and *CD-1* in control PPARα humanized mice than control wild-type mice as a baseline. This could indicate that there was already increased cell cycling going on in the control PPARα humanized mouse and could be related to the increased liver size. Treatment with WY-14,643 induced an increase in cycling genes in wild-type mice in relation to its control, but whether that induction was greater than control levels for PPARα humanized mice for *c-myc* and *CDk4* was not reported by the authors. Apoptosis genes were reported to have little difference between control PPARα humanized and wild-type mice but to have a greater response induced by WY-14,643 in humanized mice for *p53* and *p21*. There was no consistent or large change in apoptosis genes in response to exposure to WY-14,643 in wild-type mice. The increased response of apoptosis genes in PPARα humanized mice without corresponding tumor formation does not support that response as a key event in the MOA (neither does the lack of response from WY-14,643 in wild-type mice). For genes associated with PPARα peroxisomal (Acox), microsomal (CYP4a) mitochondrial fatty oxidation (Mcad) and especially malic enzyme, there was a greater response in wild-type than PPARα humanized mouse after treatment with WY-14,643. However, this is somewhat in contrast to Cheung et al. (2004), who reported increased in some genes encoding peroxisomal, mitochondrial, and microsomal fatty oxidation enzymes in the PPARα humanized mouse after treatment with WY-14,643.

The results reported by Yang et al. (2007b) use another type of "humanized" mouse to study PPARα effects. Yang et al. (2007b) used a PPARα humanized transgenic mouse on a PPAR -/- background that has the complete human PPARα (hPPARα) gene on a PAC genomic clone, introduced onto the mouse PPARα-null background and express hPPARα not only in the liver but also in other tissues. Mice were administered WY-14,643 or Fenofibrate [0.1% or 0.2% (w/w)]. The authors show a figure representing expression of the hPPARα for two mice with the tissue used for the genotyping exhibiting great variation in expression between the two cloned mice as indicated by intensity of staining. The authors state that in agreement with mRNA expression, hPPARα protein was highly expressed in the liver of $hPPAR\alpha^{PAC}$ mice to an extent similar to the mPPARα in wild-type mice. They report that following two weeks of Fenofibrate treatment, a robust induction of mRNA expression of genes encoding enzymes responsible for peroxisomal (Acox), mitochondrial (MCAD and LCAD), microsomal (CYP4A) and cytosolic

(ACOT) fatty acid metabolism were found in liver, kidney and heart of both wild-type and hPPARα^{PAC} mice indicating that hPPARα functions in the same manner as mPPARα to regulate fatty acid metabolism and associated genes. However, the authors did no measures in Fenofibrate treated animals, only WY-14,643, raising the issue of whether there was a difference in the relative mRNA expression of genes for ACOX etc. and lipids between the two peroxisomal proliferator treatments. The expression of enzymes associated with PPARα induction was presented only for mice treated with Fenofibrate. However, the lipids results were presented only for mice treated with WY-14,643. Therefore, it cannot be established that these two agonists give the same response for both parameters. Also for the enzymes, the relative expressions compared to wild-type controls, the absolute expression, and variation between animals is not reported. It appears that the peroxisomal enzyme induction by Fenofibrate is the same in the wild-type and transgenic mice. However, in Figure 4 of the paper the mice treated with WY-14,643 instead of Fenofibrate were presented for the peroxisomal membrane protein 70 (PMP70) in total liver protein gel. There appears to be more PMP70 in the transgenic mice than wild-type mice as a baseline. The PMP70 appeared to be similar after WY-14,643 treatment. However, only one gel was given and no other quantitation was given by the authors.

The authors state that "in addition WY-14,643 and Fenofibrate treatment produced similar effect to the liver specific humanized PPARα mouse line (Cheung et al 2004)." However, the results were not the same between Fenofibrate and WY-14,643 and the mouse line used by Cheung et al. had background differences in response and pathology. In one figure in the paper there appears to be a difference in background level of serum total triglyceride between the wild-type and hPPARα^{PAC} mice that the authors do not note. The power of using such few mice does not help discern any significant differences in background level of triglycerides. The authors note that WY-14,643 treatment also resulted in decreased serum triglycerides levels in hPPARα^{PAC} mice consistent with the induction of expression of genes encoding fatty acid metabolism and that the hypolipidemic effects of fibrates are generally explained by increased expression of LPL and decreased expression of apolipoprotein C- III (Apo C-III) (Auwerx et al., 1996). However, the alteration of these genes by WY-14,643 treatment was only observed in wild-type mice and not in hPPARα^{PAC} mice suggesting that the hypolipidemic effect observed in hPPARα^{PAC} mice are not through LPL and APO C-III. The authors do not note that there could be a difference in the regulation of these pathways by the transgene rather than how the normal gene is regulated and the pathways it affects. The rationale for examining this question with WY-14,643 treatment rather than with Fenofibrate treatment is not addressed by the authors, especially since the other "markers" of peroxisomal gene induction appear to be affected by Fenofibrate in the wild-type and hPPARα^{PAC} mice.

Hepatomegaly was reported to be observed in the hPPARα^{PAC} mice following two weeks of WY-14,643 treatment as revealed by the increase liver to body weight ratio compared to untreated hPPARα^{PAC} mice but to be markedly lower when compared to wild-type mice under the same treatment. Histologically, the livers of the wild-type mice treated with WY-14,643 were hypertrophic with clear eosinophilic regions. These phenotypic effects were observed in both wild-type and hPPARα^{PAC} mice. The percent liver/body weight was reported to increase from ~4% in wild-type mice to ~9% after WY-14,643 treatment and from ~4% in hPPARα^{PAC} to little less that 6% after treatment with WY-14,643. In wild-type mice treated with WY-14,643 the labeling index was 21.8% compared with 1.1% in untreated wild-type controls. In hPPARα^{PAC} mice, WY-14,643 treatment was reported to give an average labeling index of 1.0% compared with 0.8% in the untreated control hPPARα^{PAC} mice. Treatment with WY-14,643 treatment was reported to result in a marked induction in the expression of CDK4 and cyclin D1 in the livers of wild-type mice but to be unaffected hPPARα^{PAC} mice treated with WY-14,643. These data were reported to be in agreement with the liver-specific PPARα-humanized mice that showed not increase in incorporation of BrdU into hepatocytes upon treatment with WY-14,643 (Cheung et al., 2004) and further confirmed that activation of hPPARα dose not induce hepatocyte proliferation. However, the authors present a figure as an example with one liver each with no quantitation given by the authors for BrdU incorporation. It is not clear whether the pictures were taken from the same area of the liver or how representative they are. The numbers of mice were never reported for the labeling index. The data presented do suggest that there was hypertrophy and hepatomegaly in the humanized mice and but not proliferation in this particular WY,-14,643 model. Of interest would be investigation of proliferation by other peroxisome proliferators besides WY-14,643 at this necrogenic dose as it is WY-14,643 that is the anomaly to continue to induce proliferation or DNA synthesis at 2 weeks. The photomicrographs presented by the authors are so small and at such low magnification that little detail can be discerned from them. There are no portal triads or central veins to orient the reader as to what region of the liver has been affected and where if any there would be hepatocellular vacuolization.

To determine whether peroxisome proliferation occurred in the hPPARα^{PAC} mice upon administration of PPs, Yang et al. (2007b) examined by Western Blot analysis the protein levels of the major PMP70 a marker of peroxisome proliferation). After two weeks treatment of 1,000 ppm WY-14,643, induction of PMP70 was reported to be observed in the wild-type mice as well as in hPPARα^{PAC} mice. The authors suggested that this result indicates that peroxisomal proliferator treatment induced peroxisomal proliferation in hPPARα^{PAC} mice. The results of this study indicate that hepatomegaly and peroxisome proliferation occur in this humanized mouse model when treated with large concentrations of WY-14,643. Thus, these results are inconsistent with claims that peroxisome proliferators cannot cause hepatomegaly or peroxisome proliferation in humans or that humans are refractory to these effects. Like the lipid effects, they suggest a broader spectrum of effects may occur in humans and decreases the specificity of these effects as species specific. However, due to the model compound being WY-14,643 at a necrogenic dose of 1,000 ppm, the effect may not be seen in humans using the lower potency peroxisome proliferators. It would have been useful for this study to include an examination of these effects with Fenofibrate rather than WY-14,643 and then attempting to extrapolate such effects to other peroxisome proliferators. The authors often attribute the effects of peroxisome proliferators to those reactions induced by WY-14,643 and do not acknowledge that the changes induced by WY-14,643 may be different. This is especially true in regards to hepatocellular DNA synthesis in which other peroxisome proliferators can cause liver tumors without the sustained proliferation that WY-14,643 induces, especially at a necrogenic dose.

Yang et al. (2007b) report the results of induction of various genes by WY-14,643 in wild-type and hPPARα^{PAC} mice by microarray analysis followed by confirmation and quantitation by qPCR and report that more genes were induced by WY-14,643 in wild-type mice than in hPPARα^{PAC} mice. They report that

> importantly, the oncogene c-myc was not induced in hPPARα^{PAC} mice. Moreover, genes encoding cell surface proteins such as Anxa2, CD39, CD63, Ly6D, and CD24a, and several other genes such as *Cidea, Cidec, Dhrs8* and *Hsd11b* were also not induced in hPPARα^{PAC} mice. Interestingly, Sult2a1 was only induced in hPPARα^{PAC} mice and not in WT mice; this gene is also induced in human hepatocytes by PP (Fang et al., 2005). The regulation of several of these genes has previously been demonstrated through a PPARα-dependent mechanism. Additional studies will be necessary to fully explore the molecular regulatory mechanism and the functional implication associated with these differently regulated genes.

The authors do not indicate the context of how the mice were treated, whether these are pooled results, and when the samples were taken. It is assumed to be whole liver. As stated in Section E.3.2.2 above, there are several limitations for interpretations of the results such as those presented by Yang et al. (2007b) which include the lack of phenotypic anchoring for the results. The authors have shown changes from whole liver and have listed changes in genes between wild-type and humanized mice on a PPAR -/- background that in itself with bring about changes in gene expression. The authors acknowledge difficulties in determining what their reported gene changes mean.

Yang et al. (2007b) report that "activation of PPARα alters hepatic miRNA expression (Shah et al., 2007)." They report that let-7C, a miRNA critical in cell growth and shown to target c-myc, was inhibited by WY-14,643 treatment in wild-type mice and that the expression levels of both pri-let-7C and mature let-7C were significantly higher in hPPARα^{PAC} mice compared to wild-type mice. Treatment with WY-14,643 was reported to decrease the expression of Pri-let-7C and mature let-7C in wild-type mice but in hPPARα^{PAC} mice. The authors note that

> in addition, the induction of *c-myc* by WY-14,643 treatment in wild type mice did not occur in WY-14,643 treated hPPARα^{PAC} mice. This is in agreement with the previous observation in liver-specific humanized PPARα (Shah et al 2007) and further indicates the activation of human PPARα does not cause a change in hepatic miRNA and c-*my*c gene expression.

A qPCR analysis of pri-let-7C following 2 weeks WY-14,632 treatment was reported for wild-type and hPPARα^{PAC} mice (n = 3−4). There appeared to be ~20 times more let-7C expression in hPPARα^{PAC} mice than control wild mice as a baseline. The gel given by the authors showed a very small difference in wild-type mice in let-7C northern blot analysis between a control wild-type and WY-14,643-treated wild-type mouse. There appeared to be no difference in the hPPARα^{PAC} mice between control and WY-14,643 treatment and a larger stained area than the control wild-type mice. The relative c-Muc expression between the hPPARα^{PAC} mice and wild-type control mice did not correlate with changes in let-7C expression. Thus, the amount of decrease by treatment with WY-14,632 in wild-type mice appeared to be extremely small compared to the much greater baseline expression in the hPPARα^{PAC} mice. The change brought by WY-14,632 treatment in wild-type mice was a small change compared to the 20-fold greater baseline expression in the hPPARα^{PAC} mice. The authors stated that the expression of the c-Myc regulator was higher in the hPPARα^{PAC} mice indicating over regulation of cell division and an inability for hepatocytes to proliferate. However, their results showed that there was a greater difference in regulatory baseline function of the PPAR using this paradigm and this construct. Are these differences due to human PPAR or to the way PPAR was put back into PPAR -/- mouse and expected to function? If the experiment included mouse PPAR put back in this way on a null background, what would such an experiment show? Are these results representative of the PPAR or how it is now controlled and expressed? In addition, what would the study of other peroxisome proliferators besides WY-14,643 show in regard to changes in miRNA. Are these results reflective of a just the transient effect that is prolonged in a special case? As discussed in Section E.3.2.2 there are issues with microarray data in addition to the newly emerging field of miRNA arrays, which include phenotypic anchoring and whether they are from whole liver or pooled samples. The results given in this report are for relative Let-7C expression given and not absolute values. The changes in baseline Let-7C expression between the wild-type and the hPPARα^{PAC} mice did not correlate with the magnitude of difference in northern blot analysis and did not correlate at all with c-myc expression reported in this study. Thus, a direct correlation between the effect of Let-7C expression and function and effects from WY-14,643 was not supported. The relative expression was reported but the variation of baseline expression of the "PPAR controlled genes" was not. Given that one of the first figures reported a large difference between animals in expression of the human PPAR gene in the transgenic animals, how did this difference affect the results given here as relative changes downstream?

Yang et al. (2007b) conclude that the hPPARα^{PAC} mice represent the most relevant model for humans since, the tissue distribution of PPARα is similar to that observed in wild-type mice and the hPPARα in hPPARα^{PAC} mice is under regulation of its native promoter. Indeed up-regulation of hepatic mPPARα in wild-type mice by fasting was mirrored by the hPPARα in hPPARα^{PAC} mice. However, there was no demonstration that the artificial chromosome that is replicating along with other DNA is controlled sterically by the same control since it is not on the mouse genome in the same place as the native PPAR. There is also not a demonstration of how stable the baseline of PPAR DNA expression is in this mouse model—does it vary as much or more than native PPAR between mice? The authors state that

> induction of PPARα target genes for fatty acid metabolism and a decrease in serum triglycerides by PP in hPPARα^{PAC} mice indicates that hPPARα is functional in the mouse environment with respects to regulation of fatty acid metabolism. This is in agreement with the liver-specific PPARα humanized mice that also exhibit these responses (Cheung et al., 2004). Indeed the DNA binding domain of hPPARα is 100% homologous with that of the mouse suggesting that both bind to the same PPRE binding site in the promoter region of target genes. Transfection of hPPAR into murine hepatocytes increased PPs induced peroxisome proliferation related effects (Macdonald et al., 1999). These results suggest that hPPARα and mPPARα do not differ in induction of target genes with known PPRE.

However, replacement with human PPAR in the Cheung et al. model is not sufficient to prevent the same types of toxicity as seen with PPAR knockouts on the hepatocytes such as steatosis.

Yang et al. (2007b) note that

> the increased LPL and decreased expression of apo C-III are proposed to explain the hypolipidemic effects of PPS (Auwerx et al., 1996). However, hPPARα^{PAC}

> mice treated with PP exhibit lowered serum triglycerides without alteration of the expression of LPL and apo C-III. This indicates the hypolipidemic effects in rodents are mediated via other molecular regulatory mechanisms. It is also suggested that the activation of PPARα by PPs stimulates hepatic fatty acid oxidation and thereby diminishing their incorporation into triglycerides and secretion of VLDL (Froyland et al., 1997). Consistent with this idea, a robust induction of the genes encoding enzymes for fatty acid oxidation by PP in hPPARα^{PAC} mice were observed. Thus, the exact mechanism by which PPs exert their hypolipidemic effects needs reexamination.

However, the use of two different peroxisome proliferators (i.e., WY-14,643 and Fenofibrate) for two types of effects (peroxisomal and lipid) may be the cause of some paradoxes here in terms of MOA for lipid effects. The baseline differences in the hPPARα^{PAC} mice for serum total triglycerides was not explored by these authors and the small number of animals used make conclusions difficult about the magnitude of difference. The differences in baseline expression for LPL are not discernable in the graphic representation of the results.

Yang et al. (2007b) note that

> on the other hand, the difference in the affinity of ligands for the human and mouse PPARα receptor was proposed to account for the species difference. The ligand binding domain of hPPARα is 94% homologous with that of the mouse. *In vitro* transactivation assays have previously shown that WY has a higher affinity for rodent PPARα than human PPARα, while Fenofibrate has similar affinity for rodent and human PPARα (Shearer and Hoekstra, 2003; Sher et al., 1993). In the present study WY and Fenofibrate exhibit the same capacity to induce known PPARα target genes in the liver, kidney and heart in both wild-type and hPPARα^{PAC} mice.

The statement by the authors that Fenofibrate and WY-14,643 had the same affinity “as shown by this study” is not correct. The two treatments were not studied for the same enzymes or genes in the data reported in the study. Both WY-14,643 and Fenofibrate can induce PPARα targets but it was not shown to the same extent. Yang et al. (2007b) state that

> This is in agreement with the liver-specific PPARα humanized mice that also exhibit a similar capacity to induce PPARα target genes in liver by WY and Fenofibrate (Cheung et al., 2004). Thus, the ligand affinity difference between mouse and human PPARα may not be critical under the conditions of these studies.

Alternatively, these results could reflect that these studies were conducted with two different agonists with different affinities and responses due to receptor activation.

Finally, a useful comparison to make are the differences between wild-type mice, PPARα -/- mice that serve as the background for the transgenic human mouse models, and both transgenic models. The small and variable number of animals examined in these studies is readily apparent. The results of the Cheung et al. (2004) humanized mouse model and those reported for Yang et al. (2007b) show differences in the study designs including PPARα agonists studied for particular effects and results reported for similar treatments (see Table E-18).

As shown above, the effect on the PPARα -/- by the knockout included decreased triglyceride levels and slightly increased liver weight. Although treatment with WY-14,643 and Fenofibrate were reported to decrease triglyceride levels in wild-type mice, paradoxically so did knocking out the receptor. Exposures to WY-14,643 appeared to induce a slight increase and Fenofibrate a slight decrease in triglyceride levels in PPARα -/- mice but the variability of response and small number of animals in the experiments limited the ability to discern a quantitative difference in the treatments. In the study by Cheung et al. (2004) it appears that the insertion of humanized PPARα restored the baseline and treatment responses for triglyceride levels. Overall, the results reported by Yang et al. (2007b) appeared to show a lower level of triglycerides in control wild-type mice that was similar in magnitude to the treatment effect reported by Fenofibrate by Cheung et al. (2004). However, there also appeared to be restoration of this effect in the humanized mouse model of Yang et al. (2007b). In regard to DNA synthesis, both Cheung et al. (2004) and Yang et al. (2007b) only gave results for WY-14,643 and for different durations of exposure so they were not comparable. It appeared that ~60% of hepatocytes were labeled by 8 weeks of WY-14,643 treatment (Cheung et al., 2004) compared to ~20% after 2 weeks of exposure. Again this highlights the difference between using WY-14,643 as a model for the PPARα as a class at times when almost all other PPARα agonists have ceased to increase DNA synthesis or have reductions in this parameter. The background changes due to the PPARα -/- knockout were not reported so that the effects of the knockout could not be ascertained. It appeared that insertion of humanized PPARα did not result in restoration of WY-14,643 –induced DNA synthesis. The correlation with this parameter and any focal areas of necrosis were not discussed by the authors of the study. In regard to hepatomegaly, Fenofibrate and WY-14,643 appeared to both give an increase in liver weight in the humanized mouse model of Cheung et al. (2004) with little effect in the knockout mouse. For Fenofibrate there was little difference in liver weight gain in the wild-type mouse and that of the humanized mouse model of Cheung et al. (2004). However, Fenofibrate was not tested in the humanized mouse model of Yang et al. (2007b). In that model only WY-14,643 was used but there was still an increase in liver weight. Thus, in terms of effects on liver weight gain and triglyceride levels both models gave comparable results and appeared to indicate that insertion humanized PPARα would restore some of the effects of the knockout. However, the results from both experiments highlight the need for adequate numbers of animals and other PPARα agonists to be tested besides WY-14,463 at such a high dose and certainly for longer periods of time to ascertain whether such manipulations will affects carcinogenicity.

E.3.4.1.4. ***NF-κB activation.*** NF-κB activation has also been proposed as a key event in the induction of liver cancer through PPARα activation. As discussed in Sections E.3.2.6 and E.3.4.3.3, activation of the NF-κB pathway is implicated in carcinogenesis, nonspecific for a particular MOA for liver cancer, and is context dependent on its effects. Its specific actions depend on the cell type and type of agent or signal that activates translocation of the complex. NF-κB is not only involved in biological processes other than tumor induction, but also exhibits some apparently contradictory behaviors (Perkins and Gilmore, 2006). Although many studies point to a tumor-promoting function of NF-κB subunits, evidence also exists for tumor suppressor functions. NF-κB actions are associated with TNF and JNK among many other cell signaling systems and molecules and it has functions that alter proliferation and apoptosis. NF-κB activation reported in some studies may be associated with early Kupffer cell responses and be associative but not key events in the carcinogenic process. However, most assays look at total NF-κB expression in the whole liver and at the early periods of proliferation and apoptosis. The origin of the NF-κB is crucial as to its effect in the liver. For instance, hepatocyte specific deletion of IKKβ increased DEN-induced hepatocarcinogenesis but a deletion of IKKβ in both hepatocytes and Kupffer cells however, were reported to have the opposite effect (Maeda et al., 2005).

E.3.4.1.5. ***Phenotype as an indicator of a PPARα mode of action (MOA).*** As discussed previously (see Sections E.3.1.5, and E.3.1.8) FAH precede both hepatocellular adenomas and carcinomas in rodents and, in humans with chronic liver diseases that predispose them to hepatocellular carcinomas. Striking similarities in specific changes of the cellular phenotype of preneoplastic FAH are emerging in experimental and human hepatocarcinogenesis, irrespective of whether this was elicited by chemicals, hormones, radiation, viruses, or, in animal models, by transgenic oncogenes or *Helicobacter hepaticus*. Several authors have noted that the detection of phenotypically similar FAH in various animal models and in humans prone to developing or bearing hepatocellular carcinomas favors the extrapolation from data obtained in animals to humans (Bannasch et al., 2003; Su and Bannasch, 2003; Bannasch et al., 2001). In regard to phenotype by tincture Caldwell and Keshava (2006) state:

Table E-18. Comparison between results for Yang et al. (2007b) and Cheung et al. (2004)[a]

Effect	Wild type mice	PPAR -/- knockout mice	Humanized mice (liver only)	Humanized PAC mice
Triglycerides	**Cheung** **(*n* = 6−9)** Control 145 mg/mL 0.1% WY-14,643 (2 wks) 60 mg/mL 0.2% Fenofibrate (2 wks) 85 mg/mL **Yang** **(*n* = 4−6)** Control 95 mg/mL 0.1 % WY-14,643 (2wks) 55 mg/mL	**Cheung** **(*n* = 6−9)** Control 100 mg/mL 0.1% WY-14,643 (2 wks) 115 mg/mL 0.2% Fenofibrate (2 wks) 85 mg/mL	**Cheung** **(*n* = 6−9)** Control 175 mg/mL 0.1%WY-14,643 (2 wks) 60 mg/mL 0.2% Fenofibrate (2 wks) 85 mg/mL	**Yang** **(*n* = 4−6)** Control 120 mg/mL 0.1%WY-14,643 (2 wks) 75 mg/mL
BrdU incorporation	**Cheung** **(*n* = 5)** Control 1.6% 0.1% WY-14,643 (8 wks) 57.9% **Yang** **(*n* = 4−6)** Control 1.1% 0.1% WY-14,643 (2 wks) 21.8%	Not done	**Cheung** **(*n* = 5)** Control 1.6% 0.1% WY-14,643 (8 wks) 2.8%	**Yang** **(*n* = 4−6)** Control 0.8% 0.1% WY-14,643 (2 wks) 1.0%

Table E 18. Comparison between results for Yang et al. (2007b) and Cheung et al. (2004) (continued)

Effect	Wild type mice	PPAR -/- knockout mice	Humanized mice (liver only)	Humanized PAC mice
Hepatomegaly[b] **(% liver body weight ratio)**	**Cheung** **(*n* = 5−9)** Control 4% 0.1% WY-14,643 11% (2 wks) 0.2% Fenofibrate 8.5% (2 wks) **Yang** **(*n* = 4−6)** Control 4% 0.1% WY-14,643 9% (2 wks)	**Cheung** **(*n* = 5−9)** Control 5% 0.1% WY-14,643 5% (2 wks) 0.2% Fenofibrate 5.5% (2 wks)	**Cheung** **(*n* = 5−9)** Control 4.5% 0.1% WY-14,643 7% (2 wks) 0.2% Fenofibrate 7% (2 wks)	**Yang** **(*n* = 4−6)** Control 4% 0.1% WY 6% (2 wks)

[a]The ages of the humanized knockout mice are not given for Cheung et al. (2004) but are 8−10 weeks for Yang et al. (2007b).
[b]Percentages are approximate values extrapolated from figures for hepatomegaly.

> In addition, the term "basophilic" in describing preneoplastic foci or tumors can be misleading. The different types of FAH have been related to three main preneoplastic hepatocellular lineages: 1) the glycogenotic-basophilic cell lineage, 2) its xenomorphic-tigroid cell variant, and 3) the amphophilic-basophilic cell lineage. Specific changes of the cellular phenotype of the first two lineages of FAHs are similar in experimental and human hepatocarcinogenesis, irrespective of whether they were elicited by DNA-reactive chemicals, hormones, radiation, viruses, transgenic oncogenes and local hyperinsulinism as described by the first two FAHs and this similarity favors extrapolation from data obtained in animals to humans (Bannasch et al., 2003; Su and Bannasch, 2003; Bannasch et al., 2001). In contrast, the amphophilic cell lineage of hepatocarcinogenesis has been observed mainly after exposure of rodents to peroxisome proliferators or to hepadnaviridae (Bannasch et al., 2001).
>
> Bannasch (1996) describes "amphophilic" FAH and tumors induced by peroxisome proliferators to maintain the phenotype as the foci progress to tumors. They are glycogen poor from the start with increased numbers of mitochondria, peroxisomes and ribosomes. The author further states that the "homogenous basophilic" descriptions by others of foci induced by WY are really amphophilic. Agents other than peroxisome proliferators can induce "acidophilic" or "eosinophilic" (due to increased smooth endoplasmic reticulum) or glycognotic foci which tend to progress to basophilic stages (due to increased ribosomes).
>
> Tumors and foci induced by peroxisome proliferators have been suggested to have a phenotype of increased mitochondrial proliferation and mitochondrial enzymes (thyromimetic rather than insulinomimetic) (Keshava and Caldwell, 2006).

Tumors from peroxisome proliferators in Kraupp-Grasl et al. (1990) and Grasl-Kraupp et al. (1993) for rat liver tumors were characterized as weakly basophilic with some eosinophilia and as similar to the description given by Bannasch et al as amphophilic. However, a number of recent studies indicate that other "classic" peroxisome proliferators may have a different phenotype than has been attributed to the class through studies of WY-14,643. A recent study of DEHP, another peroxisome proliferator assumed to induce liver tumors through activation of the PPARα receptor, reported the majority of liver FAH to be of the first two types after a lifetime of exposure to DEHP with a dose-related tendency for increased numbers of amphophilic FAHs in rats (Voss et al., 2005). As stated previously, the MOA of DEHP-induced liver tumors in mice also appears not to be dependent on PPARα activation.

Michel et al. (2007) report the phenotype of tumors and foci in rats treated with clofibric acid at a very large dose (5,000 ppm for 20 months) and note that in controls the first type of foci to appear was tigroid on Day 264 and their incidence increased with time representing the most abundant type in this group. They report no adenomas or carcinomas after up to 607 days after giving saline injection in the control animals. DEN treatment was examined up to 377 days only with tigroid, eosinophilic and clear cell foci observed at that time. Clofibric acid was examined up to 607 days with tigroid and clear cell foci reported to be the first to appear on Day 264 no other foci class. By Day 377, there were tigroid, eosinophilic and clear cell foci but no basophilic foci reported with clofibric acid treatment and, although only a few animals were examined, 2/5 had adenomas but not carcinomas. By Day 524 all types of foci were seen (including basophilic for the first time) and there were adenomas and carcinomas in 2/5 animals. By 607 days a similar pattern was observed without adenomas but 3/6 animals showing carcinomas. Although the number of animals examined is very small, these results indicate that clofibric acid was not inducing primarily "basophilic foci" as reported for peroxisome proliferators but the first foci are tigroid and clear cell foci. Basophilic foci did not appear until Day 524 similar to control values for foci development and distribution. However, unlike controls, clofibric acid induced eosinophilic and clear cell foci earlier. This is inconsistent with the phenotype ascribed to peroxisome proliferators as exemplified by WY-14,643.

In regard to GST-π and γ-transpeptidase (GGT), Rao et al. (1986) fed 2 male F344 rats a diet of 0.1% WY-14,643 for 19 months or 3 F344 rats 0.025% Ciprofibrate for 15−19 months and reported "altered areas,"(AA) "neoplastic nodules" (NN), and hepatocellular carcinomas (HCC). For WY-14,643 treatment 107 AA, 75 NN, and 5 HCC, and for Ciprofibrate treatment 107 AA, 27 NN, and 16 HCC were identified. In the WY-14,643-treated rats, HCC, and NN were both GGT and GST-π negative (96−100%) with 87% of AA was negative for both. In Ciprofibrate-treated rats NN and HCC were negative for both markers (95%) but only 46% of AA were negative for both markers. Thus, a different pattern for tumor phenotype was reported for WY-14,643 and another peroxisome proliferator, Ciprofibrate, in this study as well.

In addition, GGT phenotype is reported not to be specific to weakly basophilic foci. GGT staining was reported to be negative in eosinophilic tumors after initiation and promotion. Kraupp-Grasl et al. (1990) note differences among PPARα agonists in their ability to promote tumors and suggest they not necessarily be considered a uniform group. Caldwell and Keshava (2006) suggest that the reports of a simple designation of "basophilic" is not enough to associate a foci as caused by peroxisome proliferators (Bannasch, 1996; Grasl-Kraupp et al.,1993; Kraupp-Grasl et al., 1990). Increased basophilia of tumors and increased numbers of carcinomas is consistent with the progressive basophilia described by Bannasch (1996), as many adenomas progress to carcinomas.

It should be noted that the amphophilic foci and tumors described by Bannasch et al. were primarily studied in rats. Morimura et al. (2006) noted that WY-14,643 induced diffusely basophilic tumors in mice and therefore, identified the WY-14,643 tumors in a way consistent with the descriptions of amphophilic tumors by Bannasch et al. The tumor induced by WY-14,643 in their humanized mouse was reported to be similar to those arising spontaneously in the mouse. However, the mouse response could differ from the rat, especially for PPARα agonists other than WY-14,643.

H-ras activation and mutation studies have attempted to assign a pattern to peroxisome proliferator-induced tumors as noted in Section E.2.3.3.2, above. However, also as noted in Section E.2.3.3.2, the genetic background of the mice used, the dose of carcinogen and the stage of progression of "lesions" (i.e., foci vs. adenomas vs. carcinomas) may affect the number of activated H-ras containing tumors that develop. Fox et al. (1990) note that tumors induced by Ciprofibrate (0.0125% diet, 2 years) had a much lower frequency of H-ras gene activation than those that arose spontaneously (2-year bioassays of control animals) or induced with the "genotoxic" carcinogen benzidine-2 HCl (120 ppm, drinking H_2O, 1 year) and that the Ciprofibrate-induced tumors were reported to be more eosinophilic as were the surrounding normal hepatocytes than spontaneously occurring tumors. Anna et al. (1994) also stated that mice treated with Ciprofibrate had a markedly lower frequency of tumors with activated H-ras but that the spectrum of mutations in tumors was similar those in "spontaneous tumors." Hegi et al. (1993) tested Ciprofibrate-induced tumors from Fox et al. (1990) in the NIH3T3 cotransfection-nude mouse tumorigenicity assay and concluded that ras protooncogene activation, were not frequent events in Ciprofibrate-induced tumors and that spontaneous tumors were not promoted with it. Stanley et al. (1994) studied the effect of MCP, a peroxisome proliferator, in B6C3F1 (relatively sensitive) and C57BL/10J (relatively resistant) mice for H-ras codon 61-point mutations in MCP-induced liver tumors (hepatocellular adenomas and carcinomas). In the B6C3F1 mice, ~24% of MCP-induced tumors had codon 61 mutations and for C57BL/10J mice ~13%. The findings of an increased frequency of H-ras mutation in carcinomas compared to adenomas in both strains of mice is suggestive that these mutations were related to stage of progression. Thus, in mice, the phenotype of tumors did not appear to be readily distinguishable from spontaneous tumors based on tincture for peroxisome proliferators other than WY-14,643, but did have more of a signature in terms of H-ras mutation and activation.

The expression of c-Jun has been used to discern TCE tumors from those of its metabolites. However, as pointed out by Caldwell and Keshava (2006), although Bull et al. (2004) have suggested that the negative expression of c-*jun* in TCA-induced tumors may be consistent with a characteristic phenotype shown in general by peroxisome proliferators as a class, there is no supporting evidence of this. While increased mitochondrial proliferation and mitochondrial enzymes (thyromimetic rather than insulinomimetic) properties have been ascribed to peroxisome proliferator-induced tumors, the studies cited in Bull et al. (2004) have not examined TCA-induced tumors for these properties.

E.3.4.1.6. ***Human relevance.*** In its framework for making conclusions about human relevance, the U.S. EPA Cancer Guidelines (U.S. EPA, 2005) asks that critical similarities and differences between test animals and humans be identified. Humans possess PPARα at sufficient levels to mediate the human hypolipidemic response to peroxisome-proliferating fibrate drugs. Fenofibrate and Ciprofibrate induce treatment related increases in liver weight, hypertrophy, numbers of peroxisomes, numbers of mitochondria, and smooth endoplasmic reticulum in cynomologous monkeys at 15 days of exposure (Hoivik et al., 2004). Given the species difference in the ability to respond to a mitogenic stimulus such as partial hepatectomy (see Section E.3.3) lack of hepatocellular DNA synthesis at this time point is not unexpected and, as Rusyn et al. (2006) note, examination at differing time point may produce differing results. It is therefore, generally acknowledged that "a point in the rat and mouse key events cascade where the pathway is biologically precluded in humans in principle cannot be identified."(Klaunig et al., 2003; NAS, 2006). Thus, from a qualitative standpoint, the effects described above are plausible in humans.

As for quantitative differences, there are two key issues. First, as stated in the Cancer Guidelines, when considering human relevance, "Any information suggesting quantitative differences between animals and humans is flagged for consideration in the dose-response assessment." Therefore, while Klaunig et al. (2003) and NAS (2006) go on to suggest that "this mode of action is not likely to occur in humans based on differences in several key steps when taking into consideration kinetic and dynamic factors," under the Cancer Guidelines, such "kinetic and dynamic factors" need to be made explicit in the dose-response assessment, and should not be part of the qualitative characterization of hazard. Second, the discussion above points to the lack of evidence supporting associations between the postulated events and carcinogenic potency. Thus, because interspecies differences in carcinogenicity do not appear to be associated with interspecies differences in postulated events, they do not provide reliable metrics with which to make inferences about relative human sensitivity.

E.3.4.2. *Other Trichloroethylene (TCE) Metabolite Effects That May Contribute to its Hepatocarcinogenicity*

While the focus of most studies of TCA has been its effects on peroxisomal proliferation, DCA has been investigated for a variety of effects that are also observed either in early stages of oncogenesis (glycogen deposition) or conditions that predispose patients to liver cancer. Some studies have examined microarray profiles in attempt to study the MOA or TCE (see Section E.3.2.2 for caveats regarding such approaches). Caldwell and Keshava have provided a review of these studies, which is provided below.

E.3.4.2.1. ***DCA effects and glycogen accumulation correlations with cancer.*** As noted previously, DCA administration has been reported to increase the observable amount of glycogen in mouse liver via light microscopy and, although to not be primarily responsible for DCA-induced liver mass increases, to be increase whole liver glycogen as much by 50% (Kato-Weinstein et al., 2001). Given that TCE and DCA tumor phenotypes indicate a role for DCA in TCE hepatocarcinogenicity (see Section E.2.3.3.2, above), Caldwell and Keshava (2006) described the correlations with effects induced by DCA that have been associated with hepatocarcinogenicity.

> A number of studies suggest DCA-induced liver cancer may be linked to its effects on the cytosolic enzyme glutathione (GST)-S-transferase-zeta. GST-zeta is also known as maleylacetoacetate isomerase and is part of the tyrosine catabolism pathway whose disruption in type 1 hereditary tyrosinemia has been linked to increased liver cancer risk in humans. GST-zeta metabolizes maleylacetoacetate (MAA) to fumarylacetoacetate (FAA) which displays apoptogenic, mutagenic, aneugenic, and mitogenic activities (Bergeron et al., 2003; Jorquera and Tanguay, 2001; Kim et al., 2000). Increased cancer risk has been suggested to result from FAA and MAA accumulation (Tanquary et al. 1996). Cornett et al. (1999) reported DCA exposure in rats increased accumulation of maleylacetone (a spontaneous decarboxylation product of MAA), suggesting MAA accumulation. Ammini et al. (2003) report depletion of the GST-zeta to be exclusively a post-transcriptional event with genetic ablation of GST-zeta causing FAA and MAA accumulation in mice. Schultz et al. (2002) report that elimination of DCA is controlled by liver metabolism via GST-zeta in mice, and that DCA also inhibits the enzyme (and thus its own elimination) with young mice being the most sensitive to this inhibition. On the other hand, older mice (60 weeks) had a decreased capacity to excrete and metabolize DCA in comparison with younger ones. The authors suggest that exogenous factors that deplete or reduce GST-zeta will decrease DCA elimination and may increase its carcinogenic potency. They also suggest that, due to suicide inactivation of GST-zeta, an assumption of linear kinetics can lead to an underestimation of the internal dose of DCA at high exposure rates. In humans, GST-zeta has been reported to be inhibited by DCA and to be polymorphic (Tzeng et al 2000; Blackburn et al., 2001, 2000). Board et al. (2001) report one variant to have significantly higher activity with DCA as a substrate than other GST zeta isoforms, which could affect DCA susceptibility.

Individuals with glycogen storage disease or with poorly controlled diabetes have excessive storage of glycogen in their livers (glycogenosis) and increased risk of liver cancer (LaVecchia et., 1994; Adami et al., 1996; Wideroff et al., 1997; Rake et al., 2002). In an animal model where hepatocytes are exposed to a local hyperinsulinemia from transplanted islets of Langerhans and the remaining tissue is hypoinsulinemic, insulin induces alterations that resemble preneoplastic foci of altered hepatocytes (FAH) and develop into hepatocellular tumors in later stages of carcinogenesis (Evert et al., 2003). A number of studies have reported suppression of apoptosis, decreases in insulin, and glycogenosis in mice liver by DCA at levels that also induce liver tumors (Bull, 2004; Bull et al., 2004; Lingohr et al., 2001). In isolated murine hepatocytes, Lingohr et al. (2002) reported DCA-induced glycogenosis was dose related, occurred at very low doses (10 μM), occurred without the presence of insulin, was not affected by insulin addition, was dependent on phosphatidylinositol 3-kinase (P13K) activity, and was not a result of decreased glycogen breakdown. The authors noted that PI3K is also known to regulate cell proliferation and apoptosis in hepatocytes, and that understanding these mechanisms may be important to understanding DCA-induced carcinogenesis. They also report insulin receptor (IR) protein levels decreased to 30% of controls in mice liver after up to 52 weeks of DCA treatment. Activation of the IR is also the principal pathway by which insulin stimulates glycogen synthetase (the rate limiting enzyme of glycogen biosynthesis). However, in DCA-induced liver tumors IR protein was elevated as well as mitogen-activated protein kinase (a downstream target protein of the IR) phosphorylation. DCA-induced tumors were glycogen poor (Lingohr et al., 2001). The authors suggest that normal hepatocytes down-regulate insulin-signaling proteins in response to the accumulation of liver glycogen caused by DCA and that the initiated cell population, which does not accumulate glycogen and is promoted by DCA treatment, responds differently from normal hepatocytes to the insulin-like effects of DCA.

Gene expression studies of DCA show a number of genes identified with cell growth, tissue remodeling, apoptosis, cancer progression, and xenobiotic metabolism to be altered in mice liver at high doses (2 g/L DCA) in drinking water (Thai et al., 2001, 2003). After 4 weeks, RNA expression was altered in 4 known genes (alpha-1 protease inhibitor, cytochrome B5, stearoyl-CoA desaturase and caboxylesterase) in two mice (Thai et al., 2001). Except for Co-A desaturase, a similar pattern of gene change was reported in DCA-induced tumors (10 tumors from 10 different mice) after 93 weeks. Using cDNA microarray in the same mice, Thai et al. (2003) identified 24 genes with altered expression, of which 15 were confirmed by Northern blot analysis after 4 weeks of exposure. Of the 15 genes, 14 revealed expression suppressed two- to fivefold and included: MHR 23A, cytochrome P450 (CYP), 2C29, CYP 3A11, serum paraoxonase/arylesterase 1, liver carboxylesterase, alpha-1 antitrypsin, ER p72, GST-pi 1, angiogenin, vitronectin precursor, cathepsin D, plasminogen precursor (contains angiostatin), prothrombin precursor and integrin alpha 3 precursor. An additional gene, CYP 2A4/5, had a twofold elevation in expression. After 93 weeks of treatment with 3.5 g/L DCA, Northern blot analyses of total RNA isolated from DCA-induced hepatocellular carcinomas showed similar alteration of expression (11 of 15). It was noted that peroxisome proliferator-activated receptor (PPAR)α and IR gene expression were not changed by DCA treatment. Genes involved in glycogen or lipid metabolism were not tested.

Although it has not been possible to determine directly whether DCA is produced from TCE at carcinogenic levels, there is indirect evidence that DCA is formed from TCE *in vivo* and contributes to liver tumor development. Pretreatment with either DCA or TCE inhibits GST-zeta while TCA pretreatment does not (Schultz et al., 2002; Bull et al., 2004). TCE treatment decreased V_{max} for DCA metabolism to 49% of control levels with a 1 g/kg TCE dose resembling effects those of 0.05 g/L DCA (Schultz et al., 2002).

E.3.4.2.2. ***Genetic profiling data for Trichloroethylene (TCE): gene expression and methylation status studies.*** Caldwell and Keshava (2006) and Keshava and Caldwell (2006) report on both genetic expression studies and studies of changes in methylation status induced by TCE and its metabolites (see Sections E.2.3.2 and E.2.3.3, above) as well as differences and difficulties in the patterns of gene expression between differing PPARα agonists. In Section E.4.2.2 (below), the effects of coexposures of DCA, TCA and Chloroform on methylation status are discussed. In particular are concerns for the interpretation of studies that employ pooling of data as well as interpretation of "snapshots in time of multiple gene changes." For the Laughter et al. (2004) study in particular, it is not clear whether transcription arrays were performed on pooled data (no data on variability between individual animals was provided and the methodology section of the report is not transparently written in this regard). The issue of phenotypic anchoring also arises as data on percent liver/body weight indicates significant variability within TCE treatment groups, especially in PPARα-null mice. For studies of gene expression using microarrays Bartosiewicz et al. (2001) used a screening analysis of 148 genes for xenobiotic-metabolizing enzymes, DNA repair enzymes, heat shock proteins, cytokines, and housekeeping gene expression patterns in the liver in response TCE. The TCE-induced gene induction was reported to be highly selective; only Hsp 25 and 86 and Cyp2a were up-regulated at the highest dose tested. Collier et al. (2003) reported differentially expressed mRNA transcripts in embryonic hearts from S-D rats exposed to TCE with sequences down-regulated with TCE exposure appearing to be those associated with cellular housekeeping, cell adhesion, and developmental processes. TCE was reported to induce up-regulated expression of numerous stress-response and homeostatic genes.

For the Laughter et al. (2004) study, transcription profiles using macroarrays containing approximately 1,200 genes were reported in response to TCE exposure. Forty-three genes were reported to be significantly altered in the TCE-treated wild-type mice and 67 genes significantly altered in the TCE-treated PPARα knockout mice. Out of the 43 genes expressed in wild-type mice upon TCE exposure, 40 genes were reported by the authors to be dependent on PPARα and included genes for CYP4a12, epidermal growth factor receptor, and additional genes involved in cell growth. However, the interpretation of this information is difficult because in general, PPARα knockout mice have been reported to be more sensitive to a number of hepatotoxins partly because of defects in the ability to effectively repair tissue damage in the liver (Shankar et al., 2003; Mehendale, 2000) and because a comparison of gene expression profiles between controls (wild-type and PPARα knockout) were not reported.

As stated previously, knockout mice in this study also responded to TCE exposure with increased liver weight, had increased background liver weights, and also had higher baseline levels of hepatocyte proliferation than wild-type mice. Nakajima et al. (2000) reported that the number of peroxisomes in hepatocytes increased by 2-fold in wild-type mice but not in PPARα knockout mice. However, TCE induced increased liver weight in both male and female wild-type and knockout mice, suggesting hepatic effects independent of PPARα activation. In regards to toxicity, after three weeks of TCE treatment (0 to1,500 mg/kg via gavage), Laughter et al. (2004) reported toxicity at the1,500 mg/kg level in the knockout mice that was not observed in the wild-type mice — all knockout mice were moribund and had to be removed from the study. Differences in experimental protocol made comparisons between TCE effects and those of its metabolites difficult in this study (see Section E.2.1.13, above).

As reported by Voss et al. (2006), dose-, time course-, species-, and strain-related differences should be considered in interpreting gene array data. The comparison of differing PPARα agonists presented in Keshava and Caldwell (2006) illustrate the pleiotropic and varying liver responses of the PPARα receptor to various agonists, but did imply that these responses were responsible for carcinogenesis.

As discussed above in Section E.3.3.5 and in Caldwell and Keshava (2006),

> Aberrant DNA methylation has emerged in recent years as a common hallmark of all types of cancers, with hypermethylation of the promoter region of specific tumor suppressor genes and DNA repair genes leading to their silencing (an effect similar to their mutation) and genomic hypomethylation (Ballestar and Esteller, 2002; Berger and Daxenbichler, 2002; Herman et al., 1998; Pereira et al., 2004; Rhee et al., 2002). Whether DNA methylation is a consequence or cause of cancer is a long-standing issue (Ballestar and Esteller, 2002). Fraga et al. (2004, 2005) reported global loss of monoacetylation and trimethylation of histone H4 as a common hallmark of human tumor cells; they suggested, however, that genomewide loss of 5-methylcytosine (associated with the acquisition of a

transformed phenotype) exists not as a static predefined value throughout the process of carcinogenesis but rather as a dynamic parameter (i.e., decreases are seen early and become more marked in later stages).

Although little is known about how it occurs, a hypothesis has also been proposed that that the toxicity of TCE and its metabolites may arise from its effects on DNA methylation status. In regard to methylation studies, many are coexposure studies as they have been conducted in initiated animals, and as stated above, some are very limited in regard to the reporting and conduct of the study. Caldwell and Keshava (2006) reviewed the body of work regarding TCE, DCA, and TCA for this issue. Methionine status has been noted to affect the emergence of liver tumors. As noted by Counts et al. (1996) a choline/methionine deficient diet for 12 months did not increase liver tumor formation in C3H/HeN mice but is tumorigenic to B6C3F1 mice. Tao et al. (2000) and Pereira et al. (2004) have studied the effects of excess methionine in the diet to see if it has the opposite effects as a deficiency (i.e., and reduction in a carcinogenic response rather than enhancement). As noted above for Tao et al. (2000), the administration of excess methionine in the diet is not without effect. The data of Tao et al. (2000) suggest that percent liver/body weight ratios are affected by short-term methionine exposure (300 mg/kg) in female B6C3F1 mice. Pereira et al. (2004) reported that very high level of methionine supplementation to an AIN-760A diet, affected the number of foci and adenomas after 44 weeks of coexposure to 3.2.g/L DCA. While the highest concentration of methionine (8.0 g/kg) was reported to decrease both the number of DCA-induce foci and adenomas, the lower level of methionine coexposure (4.0 g/kg) increased the incidence of foci. Coexposure of methionine (4.0 or 8.0 g/kg) with 3.2 g/L DCA was reported to decrease by ~25% DCA-induced glycogen accumulation, increase mortality, but not to have much of an effect on peroxisome enzyme activity (which was not elevated by more than 33% over control for DCA exposure alone). Methionine treatment alone at the 8 g/kg level was reported to increase liver weight, decrease lauroyl-CoA activity and to increase DNA methylation. The authors suggested that their data indicate that methionine treatment slowed the progression of foci to tumors. Given that increasing hypomethylation is associated with tumor progression, decreased hypomethylation from large doses of methionine are consistent with a slowing of progression. Whether, these results would be similar for lower concentrations of DCA and lower concentrations of methionine that were administered to mice for longer durations of exposure, cannot be ascertained from these data. It is possible that in a longer-term study, the number of tumors would be similar. Whether, methionine treatment coexposure had an effect on the phenotype of foci and tumors was not presented by the authors in this study. Such data would have been valuable to discern if methionine coexposure at the 4.0 mg/kg level that resulted in an increase in DCA-induce foci, resulted in foci of a differing phenotype or a more heterogeneous composition than DCA treatment alone. Finally, a decrease in tumor progression by methionine supplementation is not shown to be a specific event for the MOA for DCA-induced liver carcinogenicity.

Tao et al. (2000) reported that 7 days of gavage dosing of TCE (1,000 mg/kg in corn oil), TCA (500 mg/kg, neutralized aqueous solution), and DCA (500 mg/kg, neutralized aqueous solution) in 8-week old female B6C3F1 mice resulted in not only increased liver weight but also increased hypomethylation of the promoter regions of c-Jun and c-Myc genes in whole liver DNA (data shown for 1−2 mice per treatment). Treatment with methionine was reported to abrogate this response only at a 300 mg/kg i.p. dose with 0−100 mg/kg doses of methionine having no effect. Ge et al. (2001b) reported DCA- and TCA-induced DNA hypomethylation and cell proliferation in the liver of female mice at 500 mg/kg and decreased methylation of the c-*Myc* promoter region in liver, kidney and urinary bladder. However, increased "cell proliferation" preceded hypomethylation. Ge et al. (2002) also reported hypomethylation of the c-*myc* gene in the liver after exposure to the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (2,4-D)(1,680 ppm), dibutyl phthalate (20,000 ppm), Gemfibrozil (8,000 ppm), and WY-14,643 (50−500 ppm, with no effect at 5 or 10 ppm) after six days in the diet. Caldwell and Keshava (2006) concluded that hypomethylation did not appear to be a chemical-specific effect at these concentrations. As noted above in Section E.3.3.5, chemical exposure to a number of differing carcinogens have been reported to lead to progressive loss of DNA methylation..

Caldwell and Keshava (2006) also note similar changes in methylation after initiation and treatment with DCA or TCA.

> After initiation by N-methyl-N-nitrosourea (25 mg/kg) and exposure to 20 mmL/L DCA or TCA (46 weeks), Tao et al. (2004) report similar hypomethylation of total mouse liver DNA by DCA and TCA with tumor DNA showing greater hypomethylation. A similar effect was noted for region-2 (DMR-2) of the insulin-like growth factor-II (IGF-II) gene. The authors suggest that hypomethylation of total liver DNA and the IGF-II gene found in non-tumorous liver tissue would appear to be the result of a more prolonged activity and not cell proliferation, while hypomethylation of tumors could be an intrinsic property of the tumors. Over expression of IGF-II gene in liver tumors and preneoplastic foci has been shown in both animal models of hepatocarcinogenesis and humans, and may enhance tumor growth, acting via the over-expressed IGF-I receptor (Scharf et al., 2001; Werner and Le Roith, 2000). IGF-I is the major mediator of the effects of the growth hormone; it thus has a strong influence on cell proliferation and differentiation and is a potent inhibitor of apoptosis (Furstenberger et al., 2002). Normally, expression of IGF-II in liver is greater during the fetal period than the adult, but is over-expressed in human hepatocarcinomas due to activation of fetal promoters (Scharf et al., 2001) and loss of imprinting (Khandawala et al.,

2000). Takeda et al. (1996) report IGF-II expression in the liver is monoallelic (maternally imprinted) in the fetal period is relaxed during the postnatal period, (resulting in biallelic expression), and is imbalanced in human hepatocarcinomas (leading to restoration of monoallelic IG-II expression).

However, Bull (2004) and Bull et al. (2004) have recently suggested that hypomethylation and peroxisome proliferation occur at higher exposure levels than those that induce liver tumors for TCE and its metabolites. They report that a direct comparison in the no-effect level or low-effect level for induction of liver tumors in the mouse and several other endpoints shows that, for TCA, liver tumors occur at lower concentrations than peroxisome proliferation *in vivo* but that PPARα activation occurs at a lower dose than either tumor formation or peroxisome proliferation. A similar comparison for DCA shows that liver tumor formation occurs at a much lower exposure level than peroxisome proliferation, PPARα activation, or hypomethylation. In addition, they report that these chemicals are effective as carcinogens at doses that do not produce cytotoxicity.

E.3.4.2.3. ***Oxidative Stress.*** Several studies have attempted to study the possible effects of "oxidative stress" and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through coexposure to ethanol, have been hypothesized in itself to increase levels of "oxidative stress" as a common effect for both exposures (see Section E.4.2.4, below). Oxidative stress has been hypothesized to be the MOA for peroxisome proliferators as well, but has been found to neither be correlated with cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). As a MOA, it is not defined or specific as the term "oxidative stress" is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling.

In regard to measures of oxidative stress, Rusyn et al. (2006) noted that although an overwhelming number of studies draw a conclusion between chemical exposure, DNA damage, and cancer based on detection of 8-OHdG, a highly mutagenic lesion, in DNA isolated from organs of *in vivo* treated animals, a concern exists as to whether increases in 8-OHdG represent damage to genomic DNA, a confounding contamination with mitochondrial DNA, or an experimental artifact. As described in Section E.2.2.8, the study by Channel et al. (1998) demonstrated that corn oil as vehicle had significant effects on measures of "oxidative stress" such as TBARS. Also as noted previously (see Sections E.2.1.1 and E.2.2.11), studies of TCE which employ the i.p. route of administration can be affected by inflammatory reactions resulting from that routes of administration and subsequent toxicity that can involve oxygen radical formation from inflammatory cells.

The issues with interpretation of the Channel et al. (1998) study of TCE administered via corn oil gavage to mice have already been discussed in Section E.2.1.7, above. The TBARS results indicated suppression of TBARS with increasing time of exposure to corn oil alone with data presented in such a way for 8-OHdG and total free radical changes that the pattern of corn oil administration was obscured. It was not apparent from that study that TCE exposure induced oxidative damage in the liver.

Toraason et al. (1999) measured 8-OHdG and a “free radical-catalyzed isomer of arachidonic acid and marker of oxidative damage to cell membranes, 8-Epi-prostaglandin F2α (8epiPGF),” excretion in the urine and TBARS (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats (150−200 g) exposed to single 0, 100, 500, or 1,000 mg/kg TCE i.p. injections in Alkamuls vehicle (n = 6/group). Two sequential urine samples were collected 12 hours after injection and animals were sacrificed at 24 hours with DNA collected from liver tissues and TBARS measured in liver homogenates. The mean body weights of the rats were reported to vary by 13% but the liver weights varied by 44% after the single treatments of TCE. In contrast to the large volume of the literature that reports TCE-induced increases in liver weight, the 500 and 1,000 mg/kg exposed rats were reported to have reduced liver weight by 44% in comparison to the control values. Using this paradigm, 500 mg/kg TCE was reported to induce stage II anesthesia and a 1,000 mg/kg TCE to induce Level III or IV (absence of reflex response) anesthesia and burgundy colored urine with 2/6 rats at 24 hours comatose and hypothermic. The animals were sacrificed before they could die and the authors suggested that they would not have survived another 24 hours. Thus, using this paradigm there was significant toxicity and additional issues related to route of exposure. Urine volume declined significantly during the first 12 hours of treatment and while water consumption was not measured, it was suggested by the authors to be decreased due to the moribundity of the rats. Given that this study examined urinary markers of “oxidative stress” the effects on urine volume and water consumption, as well as the profound toxicity induced by this exposure paradigm, limit the interpretation of the study. The authors noted that because both using volume and creatinine excretion were affected by experimental treatment, urinary excretion of 8-OHdG changed significantly based on the mode of data expression. Excretion of 8epiPGF was reported to be no different from controls 12−24 hours and decreased 24 hours after TCE exposure at the two highest levels. Excretion of 8-OHdG was reported to not be affected by any exposure level of TCE and, if expressed on the basis of 24-hours, decreased. TBARS concentration per gram of liver was reported to be increased at the 500 and 1,000 mg/kg TCE exposure levels (~2−3–fold).

The effects of decreased liver size in the treated animals for this measure in comparison to control animals, was not discussed by the authors. For 8-OHdG measures in the liver and lymphocytes, the authors reported that “cost prohibited analysis of all of the tissues samples” so that a subset of animals was examined exhibiting the highest TBARS levels. The number of animals used for this determination was not given nor the data except for 500 mg/kg TCE exposure level. TCE was reported to increase 8-OHdG/dG in liver DNA relative to controls to about the same extent in lymphocytes from blood and liver (~2-fold) with the results for liver reported to be significant. The issues of bias in selection of the data for this analysis, as well as the issues already stated for this paradigm limit interpretation of these data while the authors suggest that evidence of oxidative damage was equivocal.

DCA and TCA have also been investigated using similar measures. Larson and Bull (1992) exposed male B6C3F1 mice [26 ± 3 g (SD)] to a single dose of 0, 100, 300, 1,000, or 2,000 mg/kg/d TCA or 0, 100, 300, or 1,000 mg/kg/d DCA in distilled water by oral gavage (n = 4). Fischer 344 rats (237 ± 4 g) received a single oral dose of 0, 100, or 1,000 mg/kg DCA or TCA (n = 4 or 5) TBARS was measured from liver homogenates and assumed to be malondialdehyde. The authors stated that a preliminary experiment had shown that maximal TBARS was increased 6 hours after a dose of DCA and 9 hours after a dose of TCA in mice (data shown) and that by 24 hours TBARS concentrations had declined to control values (data not shown). However, time-course information in rats was not presented and the same times used for both species, (i.e., 6- and 9-hours time periods after administration of DCA and TCA) for examination of TBARS activity. A dose of 100 mg/kg DCA (rats or mice) or TCA (mice) did not elevate TBARS concentrations over that of control liver with this concentration of TCA not examined in rats. For TCA, there was a slight dose-related increase in TBARS over control values starting at 300 mg/kg in mice (i.e., 1.68-, 2.02-, and 2.70-fold of control for 300, 1,000, and 2,000 mg/kg TCA). For DCA there were similar increases over control for both the 300 and 1,000 mg/kg dose levels in mice (i.e., 3.22- and 3.45-fold of control, respectively). For rats the 1,000 and 2,000 mg/kg levels of TCA were reported to show a statistically significant increase in TBARS over control (i.e., 1.67- and 2.50-fold, respectively) with the 300 and 1,000 mg/kg level of DCA showing similar increases but with only the 300 mg/kg-induced change statistically significant different than control values (i.e., 3.0- and 2.0-fold of control, respectively). Of note, is the report that the induction of TBARS in mice is transient and had subsided within 24 hours of a single dose of DCA or TCA, that the response in mice appeared to be slightly greater with DCA than TCA at similar doses, and that for DCA, there was similar TBARS induction between rats and mice at similar dose levels.

A study by Austin et al. (1996) appears to a follow-up publication of the preliminary experiment cited in Larson and Bull (1992). Male B6C3F1 mice (8 weeks old) were treated with single doses of DCA or TCA in buffered solution (300 mg/kg) with liver examined for 8-OHdG. The authors stated that in order to conserve animals, controls were not employed at each time point. For DCA the time course of 8-OHdG was studied at 0, 4, 6, and 8 hours after administration and for TCA at 0, 6, 8, and 10 hours after of a 300 mg/kg dose ($n = 6$). There was a statistically significant increase over controls in 8-OHdG for the 4- and 6-hour time points for DCA (~1.4- and 1.5-fold of control, respectively) but not at 8 hours in mice. For TCA, there was a statistically significant increase in 8-OHdG at 8 and 10 hours for TCA (~1.4- and 1.3-fold of control, respectively).

The results for PCO and liver weight for Parrish et al. (1996) are discussed in Section E.2.3.2.2 above for male B6C3F1 mice exposed to TCA or DCA (0, 0.01, 0.5, and 2.0 g/L) for 3 or 10 weeks ($n = 6$). The study focused on an examination of the relationship with measures of peroxisome proliferation and oxidative stress. The dose-related increase in PCO activity at 21 days (~1.5-, 2.2-, and ~4.1-fold of control, for 0.1, 0.5, and 2.g/L TCA) was reported not to be increased similarly for DCA. Only the 2.0 g/L dose of DCA was reported to induce a statistically significant increase at 21-days of exposure of PCO activity over control (~1.8-fold of control). After 71 days of treatment, TCA induced dose-related increases in PCO activities that were approximately twice the magnitude as that reported at 21 days (i.e., ~9-fold greater at 2.0 g/L level). Treatments with DCA at the 0.1 and 0.5 g/L exposure levels produced statistically significant increase in PCO activity of ~1.5- and 2.5-fold of control, respectively. The administration of 1.25 g/L clofibric acid in drinking water, used as a positive control, gave ~6−7-fold of control PCO activity at 21 and 71 days exposure.

Parrish et al. (1996) reported that laurate hydroxylase activity was reported to be elevated significantly only by TCA at 21 days and to approximately the same extent (~1.4 to 1.6-fold of control) increased at all doses tested. At 71 days both the 0.5 and 2.0 g/L TCA exposures induced a statistically significant increase in laurate hydroxylase activity (i.e., 1.6- and 2.5-fold of control, respectively) with no change reported after DCA exposure. The actual data rather than percent of control values were reported for laurate hydroxylase activity with the control values varying 1.7-fold between 21 and 71 days experiments. Levels of 8-OHdG in isolated liver nuclei were reported to not be altered from 0.1, 0.5, or 2.0 g/L TCA or DCA after 21 days of exposure and this negative result was reported to remain even when treatments were extended to 71 days of treatment. The authors noted that the level of 8-OHdG increased in control mice with age (i.e., ~2-fold increase between 71-day and 21-day control mice). Clofibric acid was also reported not to induce a statistically significant increase of 8-OHdG at 21 days, but to produce an increase

(~1.4-fold of control) at 71 days. Thus, the increases in PCO activity noted for DCA and TCA were not associated with 8-OHdG levels (which were unchanged) and, also, not with changes laurate hydrolase activity observed after either DCA or TCA exposure. Of note is the variability in both baseline levels of PCO and laurate hydrolase activity. Also of note, is that the authors report taking steps to minimize artifactual responses for their 8-OHdG determinations. The authors concluded that their data does not support an increase in steady state oxidative damage to be associated with TCA initiation of cancer and that extension of treatment to time periods sufficient to insure peroxisome proliferation failed to elevate 8-OHdG in hepatic DNA. The increased 8-OHdG at 10 weeks after Clofibrate administration but lack of 8-OHdG elevation at similar levels of PCO induction by were also noted by the authors to suggest that peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic response.

As noted above for the study of Leakey et al. (2003a) (see Section E.2.3.4), hepatic malondialdehyde concentration in ad libitum fed and dietary controlled mice did not change with CH exposure at 15 months but the dietary controlled groups were all approximately half that of the ad libitum-fed mice. Thus, while overall increased tumors observed in the ad libitum diet correlated with increased malondialdehyde concentration, there was no association between CH dose and malondialdehyde induction for either diet.

E.4. EFFECTS OF COEXPOSURES ON MODE OF ACTION (MOA)—INTERNAL AND EXTERNAL EXPOSURES TO MIXTURES INCLUDING ALCOHOL

Caldwell et al. (2008b) recently published a review of the issues and studies involved with the effects of coexposures to TCE metabolites that could be considered internal (i.e., an internal coexposure for the liver) and coexposures to metabolites and other commonly occurring chemicals that are present in the environment. As they stated:

> Human exposure to a pollutant rarely occurs in isolation. EPA's Cumulative Exposure project and subsequent National Air Toxics Assessment have demonstrated that environmental exposure to a number of pollutants, classified as potential human carcinogens, is widespread [U.S. EPA, 2006;Woodruff et al., 1998]. Interactions between carcinogens in chemical mixtures found in the environment have been a concern for several decades. Furthermore, how these interactions affect the mode of action (MOA) by which these chemicals operate and how such effects may modulate carcinogenic risk is of concern as well. Thus, an understanding of the MOA(s) of a pollutant can help elucidate its potential carcinogenic risk to humans, and can also help identify susceptible subpopulations through their intrinsic factors (e.g., age, gender, and genetic polymorphisms of key metabolic and clearance pathways) and extrinsic factors (e.g. co-exposures to environmental contaminants, ethanol consumption, and pharmaceutical use). Trichloroethylene (TCE) can be a useful example for

detailing the difficulties and opportunities for investigating such issues because, for TCE, there is both internal exposure to a "chemical mixture" of multiple carcinogenic metabolites [Chiu et al., 2006a, b] and co-exposures from environmental contamination of TCE metabolites, and from pollutants that share common metabolites, metabolic pathways, MOAs, and targets of toxicity with TCE.

Typically, ground water or contaminated waste sites can have a large number of pollutants that vary in regard to information available to support the characterization of their potential hazard, and that have differing MOAs and targets. For example, Veeramachaneni et al. (2001) reported reproductive effects in male rabbits, resulting from exposure to drinking water containing concentrations of chemicals typical of ground water near hazardous waste sites. The drinking water exposure mixture contained arsenic, chromium, lead, benzene, chloroform, phenol, and TCE. Even at 45 weeks after the last exposure, mating desire/ability, sperm quality, and Leydig cell function were subnormal. However, while the exposure levels are relevant to human environmental exposures, design of this study precludes a conclusion as to which individual toxicant, or combination of the seven toxicants, caused the effects. Thus, this study exemplifies he problems associated with studying a multi-mixture milieu. Studies of the interactions of TCE metabolites or common co-exposures that report the interactions of 2 or 3 chemicals at one time are easier to interpret.

Since EPA published its 2001 draft assessment, several approaches have been reported that include examination of tumor phenotype, gene expression, and development of physiologically-based pharmacokinetic (PBPK) models to assess possible effects of co-exposure. They attempt to predict whether such co-exposures would produce additivity of response or if co-exposure would change the nature of responses induced by TCE or its metabolites. In addition, new studies on co-exposure to DBA may help identify a co-exposure of concern. These studies may give potential insights into possible MOAs and modulators of TCE toxicity. More recent information on the toxicity of individual metabolites of TCE [Caldwell and Keshava, 2006] may be helpful in trying to identify which are responsible for TCE toxicity, but may also identify the effects of environmental co-exposures.

Recently, EPA sought advice from the National Academy of Sciences (NAS) [Chiu et al., 2006a] with the NAS charge questions including the following. (1) What TCE metabolites, or combinations of metabolites, may be plausibly involved in the toxicity of TCE? (2) What chemical co-exposures may plausibly modulate TCE toxicity? (3) What can be concluded about the potential for common drinking water contaminants such as other solvents and/or haloacetates to modulate TCE toxicity? (4) What can be concluded about the potential for ethanol consumption to modulate TCE toxicity? Thus, the understanding of the

> effects of co-exposure, in the context of MOA, is an important element in understanding the risk of a potential human carcinogen.
>
> U.S. EPA's draft TCE risk assessment [U.S. EPA, 2001] identified several factors involving co-exposure to TCE metabolites, environmental contaminants, and ethanol that could lead to differential sensitivity to TCE toxicity. Research needs identified there, as well as in previous reviews [Bull, 2000; Pastino et al., 2000], included further elucidation of the interaction of TCA and DCA in TCE-induced liver tumors and a better understanding of the functional relationships among risk factors. The complexity of TCE's potential interactions with chemical co-exposures from either common environmental co-contaminants or common behaviors such as alcohol consumption mirrors the complexity of the metabolism and the actions of TCE metabolites. Thus, TCE presents a good case study for further exploration of the effects of co-exposure on MOA.

The following sections first reiterates the findings of Bull et al. (2002) in regard to simple coexposures of DCA and TCA which can be experienced as an internal coexposure after TCE exposure. A number of studies have examined the effects of TCE or its metabolites after previous exposure to presumably genotoxic carcinogen to not only determine the effect of the coexposure on liver carcinogenicity but also to use such paradigms to distinguish between the effects of TCA and DCA. Finally, not only is TCE a common coexposure with its own metabolites, but is also a common coexposure with other solvents, and the brominated analogues of TCA and DCA. The available literature is examined for potential similarities in target and effects that may cause additional concern. The effects of ethanol on TCE toxicity is examined as well as the potential pharmacokinetic modulation of risk using recently published reports of PBPK models that may be useful in predicting coexposure effects.

E.4.1. Internal Coexposures to Trichloroethylene (TCE) Metabolites: Modulation of Toxicity and Implications for TCE Mode of Action (MOA)

Exposure to TCE will produce oxidative metabolites in the liver as an internal coexposure. As stated above, the phenotypic analysis of TCE-induced tumors have similarities to combinations of DCA and TCA and in some reports to resemble more closely DCA-induced tumors in the mouse. Results from Bull et al. (2002) are presented in Section E.2.2.22 for the treatment of mice to differing concentrations of DCA and TCA in combination and the resemblance of tumor phenotype to that of TCE. In regard to cancer dose-response, the most consistent treatment-related increase in response occurred with combinations of exposure to DCA and TCA that appeared to increase lesion multiplicity when compared to effects from individual chemicals separately. Bull et al. (2002) presented results for "selected" lesions examined for pathology characterization that suggest coexposure of 0.5 g/L DCA with either 0.5

or 2 g/L TCA had a greater than additive effect on the total number of hyperplastic nodules. In addition coexposure to 0.1 g/L DCA and 2 g/L TCA was reported to have a greater than additive effect on the total number of adenomas, but not carcinomas, induced. The random selection of lesions for the determination of potential treatment-related effects on incidence and multiplicity, rather than characterization of all lesions, increases the uncertainty in this finding.

E.4.2. Initiation Studies as Coexposures

There is a body of literature that has focused on the effects of TCE and its metabolites after rats or mice have been exposed to "mutagenic" agents to "initiate" hepatocarcinogenesis. Given that most of these "initiating agents" have many effects that are not only mutagenic but also epigenetic, that the dose and exposure paradigm modify these effects, that "initiators" can increased tumor responses alone, and the tumors that arise from these protocols are reflective of simultaneous actions of both "initiator" and "promoter," paradigms that first expose rats or mice to a "mutagen" and then to other carcinogenic agents can be described as a coexposure protocols. As stated previously, DEN and *N*-nitrosomorpholine have been reported to increase differing populations of mature hepatocytes with DEN not only being a mutagen but also able to induce concurrent hepatocyte regeneration at a high dose. Thus, the effects of the TCE or its metabolites are hard to discern from the effects of the "initiating" agent in terms of MOA. As demonstrated in the studies of Pereira et al. (1997) below, the gender also determines the nature of the tumor response using these protocols. In addition, when the endpoint for examination is tumor phenotype the consequences of tumor progression are hard to discern from the MOA of the agents using paradigms of differing concentrations, different durations of exposure, lesions counted as "tumors" to include different stages of tumor progression (foci to carcinoma), and highly variable and low numbers of animals examined. However, differences in phenotype of tumors resulting from such coexposures, like the coexposure studies cited above for just TCE metabolites, can help determine that exposure to TCE metabolites results in differing actions as demonstrated by differing effects in the presence of cocarcinogens. As stated above, Kraupp-Grasl et al. (1990) use the same approach and note differences among PPARα agonists in their ability to promote tumors suggest they should not necessarily be considered a uniform group.

E.4.2.1. *Herren-Freund et al., 1987*

The results of TCE exposure alone were reported previously (E.2.2.17) for this study. This study's focus was on the effect of TCE, TCA, DCA and Phenobarbital on hepatocarcinogenicity in male B6C3F1 mice after "initiation" at 15 days with 2.5 or 10 μg/g body weight of ethylnitrosourea (ENU) and then subsequent exposure to TCE and other chemicals in drinking water begging at 4 weeks of age (an age when the liver is already undergoing rapid growth). DCA and TCA were given in buffered solutions and sodium chloride given in the water of control animals. The experiment was reported to be terminated at 61 weeks because the "mice started to exhibit evidence of tumors." Concentrations of TCE were 0, 3 and 40 mg/L, of DCA and TCA 0, 2 and 5 g/L, and of Phenobarbital 0 and 500 mg/L. The number of animals examined in each group ranged from 16 to 32. ENU alone in this paradigm was reported to induce statistically significant increases in adenomas and hepatocellular carcinomas (39% incidence of adenomas and 39% incidence of carcinomas vs. 9 and 0% for controls) at the 10 μg/g dose (n = 23), but not at 2.5 μg/g dose (n = 22). The effects of high doses of DCA and TCA alone have already been discussed for other studies, as well as the lack of statistical power using a paradigm with so few and variable numbers of animals, the limitations of an abbreviated duration of exposure which does not allow for full expression of a carcinogenic response, and problems of volatilization of TCE in drinking water. DCA and TCA treatments at these levels (5 g/L) were reported to increase adenomas and carcinomas irrespective of ENU pretreatment and to approximately the same extent with and without ENU. TCE at the highest dose was reported to increase the number of animals with adenomas (37 vs. 9% in control) and carcinomas (37 vs. 0% in controls) but only the # of adenomas/animal was statistically significant as the number of animals examined was only 19 in the TCE group. Phenobarbital was reported to have no effect on ENU tumor induction using this paradigm.

E.4.2.2. *Parnell et al., 1986*

This study used a rat liver foci bioassay (γ-glutamyltranspeptidase, i.e., GGT) for hepatic foci after at 3 and 6 month using protocols that included partial hepatectomy, DEN (10 mg/kg) or TCA (1,500 ppm in drinking water) treatment, and then promotion with 5,000 ppm TCA (i.e., 5 g/L) for 10, 20, or 30 days and phenobarbital (500 ppm) in male S-D rats (5−6 weeks old at partial hepatectomy). The number of animals per group ranged from 4−6. PCO activities were given for various protocols involving partial hepatectomy, DEN, TCA and Phenobarbital treatments but there was no controls values given that did not have a least one of these treatments. Overall, it appeared there was a slight decrease of PCO activity in rats treated with partial hepatectomy/DEN/Phenobarbital treatments and a slight increase over other treatments for rats treated with partial hepatectomy/DEN/5,000 ppm TCA or just TCA from 2 weeks to 6 months of sampling. In regard to GGT-positive foci, the partial hepatectomy/DEN/Phenobarbital group (n = 6) was reported to have more positive foci at 3 or 6 months than rats "initiated" with TCA and PB after partial hepatectomy or partial hepatectomy/Phenobarbital treatment alone (2.05 foci/cm^2 vs. ~.05−0.10 foci/cm^2 for all other groups). The number of GGT positive foci in rats without any treatment were not studied or presented by the authors. For "promotion" protocols the number of GGT positive foci induced by the partial hepatectomy/DEN/Phenobarbital protocol at 3 and 6 months, appeared to be reduced when Phenobarbital exposure was replaced by TCA coexposure but there was no dose-response between the 50, 500 and 5,000 ppm. However, TCA treatment along with partial hepatectomy and DEN treatment did increase the levels of foci (means of 0.71−0.39 foci/cm^2 at 3 months and 1.83−2.45 foci/cm^2 at 6 months) over treatment of just partial hepatectomy and DEN (0.05 ± 0.20 foci/cm^2 at 3 months and 0.30 ± 0.39 foci/cm^2 at 6 months). For the TCA animals treated only with 5,000 ppm TCA, the number of GGT positive foci at 3 months was 0.23 ± 0.16 foci/cm^2 and at 6 months 0.03 ± 0.32 foci/cm^2 with no values for untreated animals presented. For the positive control (partial hepatectomy/DEN/Phenobarbital) the number of GGT positive foci increased from 3 to 6 months (1.65 ± 0.23 foci/cm^2 and at 6 months 7.61 ± 0.72 foci/cm^2). The authors concluded that

> although TCA is reported to cause hepatic peroxisomal stimulation in rats and mice, the results of this study indicate that it is unlikely TCA's effects are related to the promoting ability seen here. The minimal stimulation of , 10 to 20% over controls of peroxisomal associated, PCO activity in TCA exposed rats was seen only at the 5000 ppm level and only within the promotion protocol. This finding is in contrast to the promoting activity seen at all three concentrations of TCA.

E.4.2.3. *Pereira and Phelps, 1996*

The results for mice that were not "initiated" by exposure to MNU, but exposed to DCA or TCA, are discussed in Section E.2.3.2.6. However, differences in responses after initiation are useful for showing differences between single and coexposures as well as differences between DCA and TCA effects. On Day 15 of age, female B6C3F1 mice received an i.p. injection of MNU (25 mg/kg) and at 7 weeks of age received DCA (2.0, 6.67, or 20 mmol/L), TCA (2.0, 6.67 mmol, or 20 mmol/L), or NaCl continuously for 31 or 51 weeks of exposure. The number of animals studied ranged from 6 to 10 in 31-week groups and 6 to 39 in the 52-week groups. There was a "recovery group" in which mice received either 20 mmol/L DCA (2.58 g/L DCA) (n = 12) or TCA (3.27 g/L TCA) (n = 11) for 31 weeks and then switched to saline for 21 weeks until sacrifice at 52 weeks. Strengths of the study included the reporting of hepatocellular lesions as either foci, adenomas, or carcinomas and the presentation of incidence and multiplicity of each separately reported for the treatment paradigms. Limitations included the low and variable number of animals in the treatment groups.

MNU was reported to not "significantly" induce foci or altered hepatocytes, adenomas, or carcinomas at 31 (n = 10) or 51 weeks (n = 39). However, MNU did increase the incidence and number/mouse of foci, adenomas and carcinomas at the 52 week sacrifice time in comparison to saline controls, albeit at lower levels than observed in DCA or TCA cotreatments groups (e.g., 10 vs. 0% foci, 17.5 vs. 2.5% adenomas, and 10 vs. 0% incidence of carcinomas at 52 weeks for MNU-treated mice vs. saline control). Coexposure of DCA (20.0 mmol/L) for 52 weeks in MNU-treated mice increased the number of foci and hepatocellular adenomas with the authors reporting "the yield of total lesions/mouse increased as a second order function of the concentration of DCA (correlation coefficients ≥ 0.998)." TCA coexposure in MNU-treated mice was reported not to result in a significant difference in yield of foci or altered hepatocytes with either continuous 52 week or 31-week exposure, but exposures to 20.0 or 6.67 mmol/L TCA did result in increased yield of liver tumors with both exposure protocols (see below).

For TCA treatment in MNU treated mice, the incidences of foci were similar (12.5 vs. 18.2%) but the number of foci/mouse was ~3-fold greater in the cessation protocol than with continuous exposure. The incidence of adenomas was reported to be the same (~66%) as well as the number of adenomas/animal between continuous and cessation exposures. For carcinomas, there was a greater incidence for mice with continuous TCA exposure (83 vs. 36%) as well as a greater number of carcinomas/mouse (~4-fold) than for those initiated mice with cessation of TCA exposure. As noted above, the number of animals treated with TCA was low and variable (e.g., 23 mice studied at 52 weeks 20.0 mmol/L TCA, and 6 mice at 52 weeks 6.67 mmol/L TCA), limiting the ability to discern a statistically significant effect in regard to dose-response. The concentration-response relationship for tumors/mouse after 31 and 51 weeks was reported to be best represented by linear progression.

A comparison of results for animals treated with MNU and 20.0 mmol/L DCA or TCA for 31 weeks and sacrificed at 31 weeks and those which were treated with MNU and DCA or TCA for 31 weeks and then sacrificed at 52 weeks is limited by the number of animals exposed (n = 10 for 31 week sacrifice DCA or TCA, n = 12 for DCA recovery group, and n = 11 for TCA recovery group). No carcinoma data were reported for animals exposed at 31 weeks and sacrificed at 31 weeks making comparisons with recovery groups impossible for this parameter and thus, determinations about progression from adenomas to carcinomas. For the MNU and DCA-treated animals, the incidence or number of animals reported to have foci at 31 weeks was reported to be 80% but 38.5% for in the recovery group. For adenomas, the incidence was reported to be 50% for DCA-treated animals at 31 weeks and 46.2% for the recovery group. For MNU and TCA-treated animals, the incidence of foci at 31 weeks was reported to 20 and

18.2% for the recovery group. For adenomas, the incidence was reported to be 60% for the TCA-treated animals at 31 weeks and 63.6% for the recovery group. Thus, this limited data set shows a decrease in incidence of foci for the MNU and DCA-treated recovery group but no change in incidence of foci for TCA or for adenomas for DCA- or TCA-treatment between those sacrificed at 31 weeks and those sacrificed 21 weeks later. In regard to multiplicity, the number of foci/mouse was reported to be 2.80 ± 0.20 for the 31-week DCA group and 0.46 ± 0.18 for the recovery group (mean ± SEM). The number of adenomas/mouse was reported to be 1.80 ± 0.83 for the 31-week group and 0.69 ± 0.26 for the recovery group. Thus, both the number of foci and adenomas per mouse was reported to be decreased after the recovery period for MNU and DCA treated mice. Given that the number of animals with foci was decreased by half, the concurrent decrease in foci/mouse is not surprising. For TCA treatments, the numbers of foci/mouse were reported to be 0.20 ± 0.13 for the 31-week group and 0.45 ± 0.31 for the recovery group. The number of adenomas/mouse for TCA-treatment groups was reported to be 1.30 ± 0.45 for the 31-week group and 0.91 ± 0.28 for the recovery group. For the MNU and TCA-treated mice, the numbers of foci/mouse were reported to be increased and the number of adenomas/mouse reported to be slightly lower. Because carcinoma data are not presented for the 31 week group, it is impossible to determine whether the TCA adenomas regressed to foci or the TCA adenomas progressed to carcinomas and more foci apparent with increased time.

For the comparison of the numbers of foci, adenomas, or carcinomas per mouse that were reported for the mice exposed at 31 weeks and sacrificed and those exposed for 52 weeks, issues arise as to the impact of such few animals studied at 31 weeks, and the differing incidences of lesions reported for these mice on tumor multiplicity estimates. The number of animals studied who treated with MNU and 20.0 mmol/L DCA or TCA for 31 weeks and then sacrificed was *n* = 10, while the number of animals exposed to 20.0 mmol/L DCA or TCA for 52 weeks was 24 for the DCA group and 23 for the TCA group. The number of animals treated at lower concentrations of DCA or TCA were even lower at the 31-week sacrifice (e.g., *n* = 6 for MNU and 6.67 mmol/L DCA at 31 weeks) and also for the 52-week durations of exposure (e.g., *n* = 6 for MNU and 6.6.7 mmol/L TCA).

At 31 weeks, 80% of the animals were reported to have foci and 50% to have foci after 52 weeks of exposure to 20.0 mmol/L DCA and MNU treatment. Thus, similar to the "recovery" experiment, the number of animals with foci decreased even with continuous exposure between 31 and 52 weeks. For adenomas, 20.0 mmol DCA exposure for 31 weeks was reported to induce adenomas in 50% of mice and after 52 weeks of exposure to induce adenomas in 73% of mice. For TCA, the number of animals with foci was reported to be 20%

at 31 weeks and 12% at 52 weeks after exposure to 20.0 mmol/L TCA after MNU treatment and similar to the incidence of foci reported for the TCA-recovery group. For 20.0 mmol TCA, adenomas reported in 60% of mice after 31 weeks and in 67% of mice after 52 weeks of exposure and also similar to the incidence of adenomas reported for the TCA-recovery group. In regard to multiplicity, the number of foci/mouse was decreased from 2.80 ± 0.20 to 1.46 ± 0.48 between 31 weeks and 52 weeks of 20.0 mmol DCA in MNU exposed mice. The number of adenomas/mouse was reported to be increased from 1.80 ± 0.83 to 3.62 ± 0.70 between 31 weeks and 52 weeks of 20.0 mmol DCA and MNU exposed mice. For 20.0 mmol/L TCA, the number of foci/mouse was 0.20 ± 0.13 and 0.13 ± 0.7 for 31- and 52-week exposures. The number of adenomas/mouse was reported to be 1.30 ± 0.45 and 1.29 ± 0.24 for 31- and 52-week exposures. Thus, by only looking at foci and adenoma multiplicity data, there would not appear to be a change between 31 and 52-weeks. However, during progression a shift may occur such that foci become adenomas with time and adenomas become carcinomas with time. For carcinomas there was no data reported for 31-week exposure in MNU and DCA- or TCA-treated mice. However, at 52 weeks 20.0 mmol DCA was reported to induce carcinomas in 19.2% of mice and 20.0 mmol TCA to induce carcinomas in 83% of mice. The corresponding numbers of carcinomas/mouse was 0.23 ± 0.10 for 20.0 mmol/L DCA treatment and 2.79 ± 0.48 for 20.0 mmol/L TCA treatment at 52 weeks in MNU treated mice. Thus, although fewer than 20% of MNU-treated mice were reported to have foci at 20.0 mmol TCA, by 52 weeks almost all had carcinomas with ~67% also having adenomas. For DCA, many more mice had foci at 31 weeks (80%) than for TCA and by 52 weeks ~70% had adenoma with only ~20% reported to have carcinomas. The incidence data are suggestive that as these high doses of DCA and TCA, TCA was more efficient inducing progression of a carcinogenic response than DCA in MNU-treated mice.

The authors interpret the decrease in foci and adenomas between animals treated with MNU and 20.0 mmol/L DCA for 31 weeks and sacrificed and those sacrificed 21 weeks later to indicate that these lesions were dependent on continued exposure. However, the total number of lesions cannot be ascertained because carcinoma data were not reported for 31-week exposures. Carcinomas were reported in the recovery group at 52 weeks (0.15 ± 0.10 carcinomas/mouse in 15.4% of animals). Of note is that not only did the number of foci/mouse and incidence decrease between the 31-week group and the recovery group, but also between 31- and 52-weeks of continuous exposure for the MNU and 20.0 mmol/L DCA treated groups. Although derived from very few animals, the 6.67 mmol/L DCA group reported no change for foci/mouse but a decrease in the incidence of foci between 31- and 52-weeks of exposure in MNU treated mice (i.e., 0.67 ± 0.18 foci/mouse in 50% of the animals at 31 weeks and 0.50 ± 0.34 foci/mouse in 20% of mice treated for 52 weeks). The numbers of foci/mouse for both MNU-treated and untreated control mice were reported to be decreased between 31 and 51 weeks as well.

As noted in Section E.3.1.8. the number of "nodules" in humans, which may be analogous to foci and adenomas, can spontaneously regress with time rather than becoming hepatocellular carcinomas. Also as tumors get larger with progression, the number of tumors/mouse can decrease due to coalescence of tumors and difficulty distinguishing between them. While data are suggestive of a decrease in the number of adenomas/mouse after cessation of DCA exposure, the incidence data are similar between the 31-week exposure and recovery groups. Of note is that the number of carcinomas/mouse and the incidence of carcinomas was reported to be similar between the MNU-treated mice exposed continuously to 20.0 mmol/L DCA for 52 weeks and those which were treated for 31 weeks and then sacrificed at 52 weeks. Also of note is that, although incidences and multiplicities of foci and adenomas was reported to be relatively low in the 2.0 mmol/L DCA exposure groups, at 52-weeks 40% of the mice tested had carcinomas with 0.70 ± 0.40 carcinomas/mouse. This was a greater percentage of animals with carcinomas and multiplicity than that reported for the highest dose of DCA. This result suggests that the effects in regard to tumor progression, and specifically for carcinoma induction, differ between the lowest and highest doses used in this experiment. However, the low numbers of animals examined for the lower doses, 31-weeks exposures, and in the recovery group decrease the confidence in the results of this study in regard to the effects of cessation of exposure on tumor progression.

In regard to tumor phenotype, in MNU-treated female mice that were not also exposed to either DCA or TCA, all four foci and 86.7% of 15 adenomas were reported to be basophilic and 13.3% eosinophilic at the end of the 52 week-study. However, when MNU-treated female mice were also exposed to DCA the number eosinophilic foci and tumors increased with increasing dose after 52 weeks of continuous exposure. At the 20.0 mmol/L level all 38 foci examined were eosinophilic and 99% of the tumors (almost all adenomas) were eosinophilic. At the 2.0 mmol/L DCA exposure there were no foci examined but about 5 of 9 tumors examined (~2:1 carcinoma:adenoma ratio) were basophilic and the other 4 were eosinophilic. For TCA coexposure in MNU-treated mice, the 20 mmol/L TCA treatment was reported to give results of 1 of the 3 foci examined to be basophilic and 2 that were eosinophilic. For the 98 tumors examined (~2:1 carcinoma/adenoma ratio) 71.4% were reported to be basophilic and 28.6% were eosinophilic. At the 2.0 mmol/L TCA exposure level, the 2 foci examined were reported to be basophilic while the 6 tumors (all adenomas) were reported to be 50% eosinophilic and 50% basophilic. Thus, after 52 weeks female mice treated with MNU and a high dose of DCA had eosinophilic foci and adenomas and those treated with the high dose of TCA had a mixture of basophilic and eosinophilic foci and tumors with a 3:1 ratio of tumors (mostly carcinomas) being basophilic. At the lower doses of either DCA or TCA the tumors tended to be mostly carcinomas for DCA and adenomas for TCA but both were ~50% basophilic and 50% eosinophilic. The tumors observed from MNU treatment alone were all adenomas and mostly 87% basophilic. Thus, not only did treatment concentrations of DCA and TCA give a different result for tumor multiplicity and incidence, but also for tumor phenotype in MNU treated female mice. Eosinophilic foci and tumors were reported to be consistently GST-π positive while basophilic lesions "did not contain GST-π, except for a few scattered cells or very small area comprising less than 5% of the tumor."

Thus, exposure to either DCA or TCA increased incidence and number of animals with lesions (foci, adenomas, or carcinomas) in MNU- versus nontreated mice (see Section E.2.3.2.6, above). These results suggest that the pattern of foci, adenoma and carcinoma incidence, multiplicity, and progression appeared to differ between TCA and DCA in MNU-treated female mice. However, the low and variable number of animals used in this study, make quantitative inferences between DCA and TCA exposures in "initiated" animals, problematic.

E.4.2.4. *Tao et al., 2000*

The source of liver tumors for this analysis was reported to be the study of Pereira and Phelps (1996). Samples of liver "tumors" and "noninvolved" liver was homogenized for protein expression for c-Jun and c-Myc and therefore, contained homogeneous cell types for study. The term "liver tumors" was not defined so it cannot be ascertained as to whether the lesions studied were altered foci, hepatocellular adenomas, or carcinomas. Liver tissues were reported to be frozen prior to study which raises issues of m-RNA quality. Although this study reports that there were no MNU-induced "tumors" the original paper of Pereira and Phelps (1996) reports that there were four foci and 15 adenomas in MNU-only treated mice. The authors reported no difference in c-Jun and c-Myc m-RNA from DCA or TCA-induced tumors from mice "initiated" with MNU. DNA methyltransferase was reported to be decreased in noninvolved liver in MNU-only treated mice in comparison to that from TCA- and DCA-treated mice. For a comparison between noninvolved liver and tumors, tumors were reported to have a greater level than did noninvolved liver.

E.4.2.5. *Lantendresse and Pereira, 1997*

This study used the tumors from Pereira and Phelps (1996), except for the MNU-treated only groups and those groups treated with either DCA or TCA but not MNU initiation, to further study various biomarkers. The omissions were cited as to be due to insufficient tissue. For immunohistochemical evaluation of the molecular biomarkers other than GST-π, liver specimens from 7 MNU/20.0 mmol DCA- (i.e., 2.58 g/L DCA) treated and 6 MNU/20.0 mmol TCA - (i.e., 3.27 g/L TCA) treated female mice randomly selected. For GST-π, the number of animals from which lesion specimens were derived, was 24 MNU/DCA-treated and 23 MNU/TCA-treated mice. The DCA treated mice were reported to have 1−9 lesions/mouse and TCA treated mice 1−3 lesions/mouse. The number of lesions examined for each biomarker varied greatly. For TCA-induced foci, no foci were examined for any biomarker except 3 lesions for GST-π, while for DCA 12−15 foci were examined for each biomarker and 38 lesions examined for GST-π. Similarly for TCA-induced adenomas, there were 8−10 lesions examined for all biomarkers with 32 lesions examined GST-π, while for DCA 12 lesions for all biomarkers with 94 lesions examined for GST-π. Finally, for TCA-induced carcinomas there were 3−4 lesions examined per group with 64 lesions examined for GST-π, while for DCA-induced carcinomas there were no lesions examined for any biomarker except 3 examined for GST-π. The biomarkers used were: GST-π, TGF-α, TGF-β, *c-Jun*, *c-Fos*, *c-Myc*, cytochrome oxidase CYP2E1, and cytochrome oxidase CYP4A1.

MNU/DCA treatment was reported to produce "predominantly eosinophilic lesions" with

> in general, the hepatocytes of DCA-promoted foci and tumors were less pleomorphic and uniformly larger and had more distinctive cell borders than the hepatocytes in lesions caused by TCA. Parenchymal hepatocytes of DCA-promoted mice were uniformly hypertrophied, with prominent cell borders, and the cytoplasm was markedly vacuolated, which was morphologically consistent with the previous description of glycogen deposition in these lesions. In contrast, TCA-promoted proliferative lesions tended to be basophilic, as previously reported, and were composed of hepatocytes with less distinct cell borders, slight cytoplasmic vacuolization, and greater variability in nuclear size and cellular size.

The hepatocytes of altered foci and hepatocellular adenomas from MNU-treated female mice also treated with DCA were reported to stain positively for TGF-α, c-Jun, c-Myc, CYP2E1, CYP4A1, and GST-π. The authors do not present the data for foci and adenomas separately but as an aggregate and as the number of lesions with <50% cells stained or the number of lesions with >50% cells stained either "minimally to mildly" or "moderately to densely" stained. Because no carcinomas for DCA were examined and especially because no foci for TCA analyses were included in the aggregates, it is difficult to compare the profile between TCA and DCA exposure in initiated animals and to separate these results from the effects of differences in tumor progression. Thus, any differences seen in these biomarkers due to progression from foci to adenoma in DCA-induced lesions or from progression of adenoma to carcinoma in TCA-induce lesions, was lost. If the results for adenomas had been reported separately, there would have been a common stage of progression from which to compare the DCA and TCA effects on initiated female mice liver tumors. For DCA-induced “lesions” (~50% foci and ~50% adenomas), most lesions had >50% cells staining with moderate to dense levels for TGF-α, and CYP2E1, CYP4A1, and GST-π and most lesions had <50% cells staining for even minimally to mild staining for TGF-β and *c-Fos*. For *c-Jun* and *c-Myc* the aggregate DCA-induced “lesions” were heterogeneous in the amount of cells and the intensity of cell staining for these biomarkers in MNU-treated female mice.

For the TCA “lesions” (~60% adenomas and ~30% carcinomas) the authors note that

> in general, the hepatocytes of tumors promoted by TCA demonstrated variable immunostaining. With the exception of c-Jun, greater than 50% of the hepatocytes in TCA lesions were essentially negative or stained only minimally to mildly for the protein biomarkers studies. In some instances, particularly in TCA-promoted tumors, there was regional staining variability within the lesions, including immunoreactivity for c-Jun and c-Myc proteins, consistent with clonal expansion or tumor progression.

As stated above, the term “lesion” refers to foci and adenomas for DCA but for adenomas and carcinomas for TCA making inferences as to differences in the actions of the two compounds through the comparisons of biomarkers confounded by the effects of tumor progression. The largest differences in patterns between TCA induced “lesions” and those by DCA appeared to be TGF-α (with no lesions having >50% cells stained mildly or moderately/densely for TCA-induced lesions), CYP2E1 (with few lesions having >50% stained moderately/densely for TCA-induced lesions), CYP4A1 (with no lesions having >50% stained mildly or moderately/densely for TCA-induced lesions), and GST-π (with all lesions having <50% cells stained even mildly for TCA-induced lesions). However, as shown by these data, while the “lesions” induced by TCA and DCA had some commonalities within each treatment, there was heterogeneity of lesions produced by both treatments in female mice already exposed to MNU. Overall, the tumor biomarker pattern suggests differences in the effects of DCA and TCA through differences in tumor phenotype they induce as coexposures with MNU treated female mice.

The authors note that nonlesion parenchymal hepatocytes in DCA–treated initiated mice stained mostly negative for CYP2E1 and CYP4A1, while in TCA-treated mice staining patterns in parenchymal nonlesions hepatocytes were centrilobular for CYP2E1 and panlobular for CYP4A1 (a pattern for CYP4A1 that is opposite of that found in the TCA-induced lesions).

E.4.2.6. *Pereira et al., 1997*

This study used a similar paradigm as that of Pereira and Phelps (1996) to study coexposures of TCA and DCA to female B6C3F1 mice already exposed to MNU. At 15 days the mice received 25 mg/kg MNU and starting at 6 weeks of age neutralized solutions of either 0, 7.8, 15.6, 25.0 mmol/L DCA (n = 30 for control and 25 mmol/L DCA and n = 20 for 7.8 and 15.6 mmol/L DCA), 6.0 or 25.0 mmol/L TCA (n = 30 for 25.0 mmol/L TCA and n = 20 for 6.0 TCA), or combinations of DCA and TCA that included 25.0 mmol/L TCA + 15.6 mmol/L DCA (n = 20), 7.8 mmol/L DCA + 6.0 mmol/L TCA (n = 25), 15.6 mmol/L DCA + 6.0 mmol/L TCA (45), 25.0 mmol/L DCA + 6.mmol/L TCA (n = 25). The corresponding concentrations of DCA and TCA in g/L is 25 mmol = 3.23 g/L, 15.6 mmol = 2.01 g/L and 7.8 mmol = 1.01 g/L DCA and 25 mmol = 4.09 g/L and 6.0 mmol = 0.98 g/L TCA. Accordingly, the number of animals at the beginning of the study varied between 20 and 45. At terminal sacrifice (after 44 weeks of exposure) the numbers of animals examined were less with the lowest number examined to be 17 mice in the 7.8 mmol/L DCA group and the largest to be 42 in the 15.6 mmol/L DCA + 6.0 mmol/L TCA exposed group.

The authors reported that only a total of eight hepatocellular carcinomas were found in the study (i.e., 25.0 mmol/L DCA induced 3 carcinomas, 7.8 mmol DCA + 6.0 mmol TCA induced one carcinoma, and 25.0 mmol/L TCA induced 4 carcinomas). Thus, they presented data for foci/mouse, and adenomas/mouse and their sum of both as "total lesions." The incidences of lesions (i.e., how many mice in the groups had lesions) were not reported. The shortened duration of exposure (i.e., 44 weeks), the omission of carcinomas from total "lesion" counts (precluding consideration of progression of adenomas to carcinomas), the lack of reporting of tumor incidences between groups, and the variable and low numbers of animals examined in each group make quantitative inferences regarding additivity of these treatments difficult. MNU treated mice did have a neoplastic response, albeit low using this paradigm. For mice that were only exposed to MNU (n = 30 at terminal sacrifice) the mean number of foci, adenomas and "lesions" per mouse were 0.21, 0.07 and 0.28, respectively. No data were given for mice without MNU treatment but few lesions would be expected in controls. Pereira and Phelps (1996) reported that saline-only treatment in 40 female mice for 51 weeks resulted in 0% foci, 0.03 adenomas/mouse in 2.5% of mice, and 0% carcinomas. In general, it appeared that the numbers of foci, adenomas and the combination of both reported as "lesions" per mouse that would have been predicted by the addition of multiplicities given for DCA, TCA, and MNU treatments alone, were similar to those observed as coexposure treatments. The largest numbers of foci and adenomas/mouse were reported for the 25.0 mmol/L DCA and 6.0 mmol/L TCA treatments in MNU treated mice (mean of 6.57 "lesions"/mouse) with the lowest number reported for 7.8 mmol/L DCA and 6 mmol/L TCA (mean of 1.16 "lesions"/mouse).

The authors reported that the foci of altered hepatocytes were predominantly eosinophilic in DCA-treated female mice initiated with MNU, while those observed after MNU and TCA treatment were basophilic. MNU treatment alone induced 4 basophilic and 2 eosinophilic foci, and 2 basophilic adenomas. MNU and DCA treatment was reported to produce only eosinophilic foci and adenomas at the 25.0 mmol/L DCA exposure level. At the 7.8 mmol/L DCA level of treatment in MNU-treated mice, 2 foci were basophilic, 4 were eosinophilic and the 1 adenoma observed was reported to be eosinophilic. Thus, the concentration of exposure appeared to alter the tincture of the foci observed after MNU and DCA exposure using this paradigm. In this study, MNU and TCA treatment was reported to induce foci and adenomas that were all basophilic at both 25.0 mmol/L TCA and 6.0 mmol/L TCA exposures. After 7.8 mmol/L DCA + 6.0 mmol/L TCA exposure, 2/23 foci were basophilic and 21/23 foci were reported to be eosinophilic while all 4 adenomas reported for this group were eosinophilic.

Irrespective of treatment, eosinophilic foci for were reported to be GST-π positive and basophilic foci to be GST-π negative. An exception was the 4 carcinomas in the group treated with 25 mmol/L TCA which were reported to be predominantly basophilic but contained small areas of GST-π positive hepatocytes.

It should be noted that the increased dose (up to 3.23 g/L DCA and 4/09 g/L TCA) raises issues of toxicity and effects on water consumption as other studies have noted toxicity at highly doses of DCA and TCA. The use of an abbreviated duration of exposure in the study raises issues of sensitivity of the bioassay at the lower doses used in the experiment. In particular, was enough time provided to observe the full development of a tumor response? Finally, a question arises as what can be concluded from the low numbers of foci examined in the study and the affect of such low numbers on the ability to discern differences in these foci by treatment. As with Pereira and Phelps, there appeared to be a difference the nature of the response induced by coexposure of MNU to relatively high versus low DCA concentrations. Of note is that while this experiment reported no hepatocellular carcinomas at the lowest dose of DCA at 44 weeks (7.8 mmol DCA), Pereira and Phelps (1996) reported that in 9 mice treated with MNU and 2.0 mmol DCA for 52 weeks, there were no foci but 20% of mice had adenomas (0.20 adenomas/mouse) and 40% of mice had carcinomas (0.70 carcinomas/mouse).

These results suggest that DCA coexposure affects TCA-induced lesions. The authors concluded that mixtures of DCA and TCA appear to be at least additive and likely synergistic and similar to the pathogenesis of DCA.

E.4.2.7. *Tao et al., 1998*

The focus of this study was an examination of tumors resulting from MNU and DCA or TCA exposure in mice with the source of tumors was reported to be the study of Pereira et al. (1997). Thus, similar concerns discussed above for that study paradigm are applicable to the results of this study. The authors stated that there were also two recovery groups in which exposure was terminated 1 week prior to euthanization at Week 44. The Pereira et al. (1997) study does not report a cessation group in the study. In this study the number of animals treated in the cessation group, the incidences of tumors in the mice, and the number of tumors examined were not reported. Another group of female B6C3F1 mice (7−8 weeks old) were reported to not be administered MNU but given 25 mmol/L DCA (3.23 g/L DCA), 25 mmol TCA (4.09 g/L TCA), or control drinking water for 11 days ($n = 7$).

Hepatocellular adenomas in DCA-treated mice, adenomas and carcinomas in TCA-treated mice were reported to be analyzed for percent-5-methylcytosine in the DNA of tumor tissues. The levels of 5-methylcytosine in liver DNA of mice administered DCA or TCA for 11 days were reported to be reduced in comparison to control tissues (reduced to ~36% of control for DCA and ~41% of control for TCA with the control value reported to be ~3.5% of DNA methylated). The number of animals examined was reported to be 7−10 animals per group.

For control liver from mice that had received MNU but not DCA or TCA, and noninvolved liver after 44 weeks of exposure to either, the levels of 5-methylcytosine were similar and not different from the ~3.5% of DNA methylated in untreated mice in the 11-days experiment. Thus, initial decreases in methylated DNA shown by exposure to DCA or TCA alone for 11 days, were not observed in "noninvolved" liver of animals exposed to either DCA or TCA and MNU.

In regard to tumor tissues, the level of 5-methylcytosine in DNA of hepatocellular adenomas receiving DCA and MNU was reported to be decreased by 36% in comparison to noninvolved liver from the same animals. When exposure to DCA was terminated for 1 week prior to sacrifice the level of 5-methylcytosine in the adenomas was reported to be higher and no longer differed statistically from the noninvolved liver from the same animal or liver from control animals only administered MNU. The number of samples was reported to be

9−16 samples without identification as to how many samples were used for each tumor analysis or how many animals provided the samples (i.e., were most of the adenomas from on animal?)

For TCA the 5-methylcytosine level was reported to be reduced by 40% in hepatocellular adenomas and 51% reduction in hepatocellular carcinomas in comparison to noninvolved liver from the same animals. These levels were also reported to be less than that the control animals administered only MNU. Termination of exposure to TCA 1 week prior to sacrifice was reported to not produce a statistically significant change in the level of 5-methylcytosine in either adenomas or carcinomas. The levels of 5-methylcytosine were reported to be lower in carcinomas than adenomas (~20% reduction) and to be lower in the "recovery" carcinomas than continuous carcinomas (~25%) but were not reported as statistically significant. The results are reported to have been derived from 8−16 "samples each." Again information on the number of animals with tumors, whether the tumors were from primarily from one animal, and which DNA results are from 8 versus 16 samples, was not provided by the authors. Given that Pereira et al. (1997), the source for material of this study, reported that treatment of MNU and 25.0 mmol/L TCA treatment for 44 weeks induced only 4 carcinomas, a question arises as to how many carcinomas were used for the 44-week 5-methylcytosine results in this study for carcinomas (i.e., how can 8−16 samples arise from 4 carcinomas?). In addition, a question arises as to whether there was a difference in tumor-response in those animals with and without one week of cessation of exposure which cannot be discerned from this report. The use of highly variable number of samples between analysis groups and lack of information as to how many tumors were analyzed adds uncertainty to the validity of these findings. There did not appear to be a difference in methylation activity from short-term exposure to either DCA or TCA alone in whole liver DNA extracts. However, the authors conclude that the difference in methylation status between tumors resulting from MNU and DCA or TCA exposures supports differences in the action between DCA and TCA.

E.4.2.8. *Stauber et al., 1998*

In this study, 5−8 week old male B6C3F1 mice were used for isolation of primary hepatocytes which were subsequently isolated and cultured in DCA or TCA. In a separate experiment 0.5 g/L DCA was given to mice as pretreatment for 2 weeks prior to isolation. The authors note that and indication of an "initiated cell" is anchorage-independent growth. DCA and TCA solutions were neutralized before use. The primary hepatocytes from 3 mice per concentration were cultured for 10 days with DCA or TCA colonies (8 cells or more) determined in quadruplicate. The levels of DCA used were 0, 0.2, 0.5 and 2.0 mM DCA or TCA. At concentrations of 0.5 mM or more DCA and TCA both induced an increase in the number of colonies that was statistically significant and increased with dose with DCA giving a slightly greater effect. The authors noted that concentrations greater than 2.0 mM were cytotoxic but did not show data on toxicity for this study.

Of great interest is the time-course experiment from this study in which the number of colonies from DCA treatment *in vitro* peaked by 10 days and did not change through days 15−25 at the highest dose. For the lower concentrations of DCA, increased time in culture induced similar peak levels of colony formation by days 20−25 as that reached by 10 days at the higher dose. Therefore, the number of colonies formed was independent of dose if the cells were treated long enough *in vitro*. The number of colonies that formed in control hepatocyte cultures also increased with time but at a lower rate than those treated with DCA (2.0 mM DCA gave ~2-fold of control by 25 days of exposure to hepatocytes in culture). However, the level reached by cells untreated in tissue culture alone by 20 days was similar to the level induced by 0.5 mM DCA by 10 days of exposure. This finding raises the issue of what these "colonies" represent as tissue culture conditions alone transform these cells to what the authors suggest is an "initiated" state. TCA exposure was not tested with time to see if it had a similar effect with time as did DCA.

At 10 days, colonies were tested for c-Jun expression with the authors noting that "colonies promoted by DCA were primarily c-Jun positive in contrast to TCA promoted colonies that were predominantly c-Jun negative." For colonies that arose spontaneously from tissue culture conditions, 10/13 (76.9%) were reported to be c-Jun +, those treated with DCA 28/34 (82.3%) were c-Jun +, and those treated with TCA 5/22 (22.7%) were c-Jun +. These data show heterogeneity in cell in colonies although more were c-Jun + with DCA than TCA. The number of colonies reported in the c-Jun labeling results represent sums between experiments and thus, present total numbers of the control and the of colonies derived from doses of DCA and TCA at 0.2 to 2.0 mM at 10 days. Thus, changes in colony c-Jun+ labeling due to increasing dose cannot be determined. The authors reported that with time (24, 48, 72, and 96 hours) of culture conditioning the number of c-Jun+ colonies was increased in untreated controls. DCA treatment was reported to delay the increase in c-Jun+ expression induced by tissue culture conditions alone in untreated controls. TCA treatment was reported to not affect the increasing c-Jun+ expression that increased with time in tissue culture. In this instance, tissue culture environment alone was shown to transform cells and can be viewed as a "coexposure." DCA pretreatment *in vivo* was reported to increase the number of colonies after plating which reached a plateau at 0.10 mM and gave changes as at low a concentration of 0.02mM DCA administered *in vitro*. The background level of colony formation varied between controls (i.e., 2-fold different in pretreatment experiments and nonpretreatment experiments).

Therefore, although the number of colonies was greater for pretreatment with DCA, the magnitude of difference over the control level was the same after DCA treatment *in vitro* with and without pretreatment.

The authors presented a comparison of "tumors" from Stauber and Bull (1997) and state that DCA tumors were analyzed after 38 weeks of treatment but that TCA tumors were analyzed after 52 weeks. They note that 97.5% of DCA-induced "tumors" were c-Jun + while none of the TCA-induced "tumors" were c-Jun +. The concentrations used to give tumors *in vivo* for comparison with *in vitro* results were not reported. What was considered to be "tumors" from the earlier report for this analysis was also not noted. Stauber and Bull (1997) reported results for combination of foci and tumors raising issues as to what was examined in this report. The authors stated that because of such short time, no control tumors results were given. The short and variable time of duration of exposure increases the possibility of differences between the *in vivo* data resulting from differences in tumor progression as well as a decreased ability by the shortened time of observation for full expression of the tumor response.

E.4.3. Coexposures of Haloacetates and Other Solvents

As noted by Caldwell et al. (2008b), drinking water exposure data suggest coexposure of TCE and its haloacetic acid metabolites, TCA and DCA, is not an uncommon event as DCA and TCA are the two most abundant haloacetates in most water supplies (Weisel et al., 1999; Boorman et al., 1999). Dibromoacetic acid (DBA) concentrations have also been reported to range up to approximately 20 μg/L in finished water and distribution systems (Weinberg et al., 2002). Caldwell et al. (2008b) have also noted that coexposure in different media also occurs with solvents like perchloroethylene (PERC) that may share some MOAs, targets of toxicity, and common metabolites that can therefore, potentially affect TCE health risk (Wu and Schaum, 2000). Some of the information contain in the following sections have been excerpted from the discussions by Caldwell et al. (2008b) regarding the implications for the risk of TCE exposure as modulated by coexposures to haloacetates and other solvents that have been studied and reported in the literature.

E.4.3.1. *Carbon tetrachloride, Dichloroacetic Acid (DCA), Trichloroacetic Acid (TCA): Implications for Mode of Action (MOA) from Coexposures*

Studies of specific combinations of TCE and chemicals colocated in contaminated areas have been reported by Caldwell et al. (2008b). For carbon tetrachloride

> Pretreatment with TCE in drinking water at levels as low as 15 mM for three days has been reported to increase susceptibility to liver damage to subsequent exposure to a single IP injection of 1 mM/kg carbon tetrachloride (CCl_4) in Fischer 344 rats [Steup et al., 1991]. Potential mechanistic explanations for this observation included altered metabolism, decreased hepatic repair capability, decreased detoxification ability, or combination of one or more of the above activities. Simultaneous administration of an oral dose of TCE (0.5ml/kg) has also been reported to increase the liver injury induced by an oral dose of 0.05 ml/kg CCl_4 [Steup et al., 1993]. The authors suggested that TCE appeared to impair the regenerative activity in the liver, thus leading to increased damage when CCl_4 is given in combination with TCE.

As discussed above in Section E.4.2, initiation studies are in themselves a coexposure. The study of Bull et al. (2004) is included here as it not only used a coexposure of vinyl carbamate with TCE metabolites, but also used carbon tetrachloride as a coexposure as well. The rationale for this approach was that coexposure of TCE (and therefore, to its metabolites) and CCl_4 are likely to occur as they are commonly found together at contaminated sites. Bull et al. (2004) hypothesized that modification of tumor growth rates is an indication of promotion rather than effects on tumor number, and that by studying tumor growth rates they could classify carcinogens by their MOAs. B6C3F1 male mice were initiated with vinyl carbamate (3 mg/kg) at 2 weeks of age and then treated with DCA, TCA, CCl_4, (0.1, 0.5, or 2.0 g/L for DCA and TCA; 50, 100 or 500 mg/kg CCL_4 in 5% Alkamuls via gavage) in pair-wise combinations of the three for 18 to 36 weeks. The exposure level of CCL_4 to 5, 20 and 50 mg/kg was reported to be reduced at Week 24 due to toxicity for CCl_4. The number of mice in each group was reported to be 10 with the study divided into 5 segments. There were evidently differences between treatment segments as the authors state that "because of some significant quantitative differences in results that were obtained with replicate experiments treated in different time frames, the simultaneous controls have been used in the analysis and presentation of these data." As with Bull et al. (2002), the interpretation of the results of the study is limited by a low number of animals per group, short duration time of exposure and limited examination and reporting of results. For example, a sample of 100 out of the 8,000 lesions identified in the study was examined to verify that the general descriptor of neoplastic and nonneoplastic lesion was correctly labeled with "tumors" describing a combination of hyperplastic nodules, adenomas, and carcinomas. No incidence data were reported by the authors, but general lesion growth information included mean lesion volume and multiplicity of lesions (numbers of lesions/mouse). Using these reported indices, there appeared to be differences in treatment-related effects.

As discussed in Caldwell et al. (2008b):

> Each treatment was examined alone and then in differing combinations with each other. Mice initiated with vinyl-carbamate, but without further exposure to the other toxicants, were reported to have a few lesions that were of small size during the examination period (20−36 weeks). At 30 weeks of CCl4 exposure, there was a dose-related response reported for multiplicity but mean lesion size was smaller at the highest dose in initiated animals. At 36 weeks, DCA exposure was reported to increase multiplicity at the two highest exposure levels and increased lesion size at all levels compared to initiated-only animals. However, at a similar level of induction, multiplicity and mean size of those lesions resulting from DCA treatment were reported to be much smaller in comparison with CCl4 treatment (i.e., a 20-fold difference for lesion volume). At 36 weeks, treatments with the same concentration of TCA or DCA induced similar multiplicity, but the mean lesion volume was reported to be approximately 4-fold greater in tumors induced by DCA as compared to TCA, and in animals treated with DCA multiplicity had reached a plateau by 24 weeks rather than 36 for those treated with TCA.

Thus, using multiplicity of lesions and lesion volume as indicators of differences in MOA, exposure to CCl_4, DCA, and TCA appeared to produce distinct differences in results in animals previously treated with vinyl carbamate.

As discussed in Caldwell et al. (2008b):

> Simultaneous coexposure of differing combinations of CCl_4, DCA, and TCA were reported to give more complex results between 24 and 36 weeks of observation but to show that coexposure had effects on lesion multiplicity and volume in initiated animals. At 36 weeks, TCA coexposure appeared to reduce the lesion volume of either DCA- or CCl_4-induced lesions after vinyl carbamate treatment. Similarly, DCA coexposure was reported to reduce the lesion volume of either TCA- or CCl_4-induced lesions when each was given alone after vinyl carbamate treatment. With regard to multiplicity, TCA coexposure was reported to reduce DCA-induced multiplicity only at the lowest dose of TCA while coexposure with DCA increased multiplicity of CCl_4-induced lesions at all exposure levels. At 24 weeks, there appeared to be little effect on mean lesion volume by any of the coexposures but DCA coexposure decreased multiplicity of TCA-induced lesions (up to 3-fold) while TCA treatment slightly increased the number of CCl_4-induced multiplicity (1.6-fold). This study confirms that short duration of exposure to all three of these chemicals can cause lesions in already exposed to vinyl carbamate, and suggests that combinations of these agents differentially influence lesion number and growth rates. The authors have interpreted their results to indicate differences in MOA between such treatments. However, the limitations of the study limit conclusions regarding how such coexposure may be able to affect toxicity and tumor induction and what the MOA is for each of these agents. This

is especially true at lower and more environmentally relevant concentrations given for longer durations to uninitiated animals.

E.4.3.2. *Chloroform, Dichloroacetic Acid (DCA), and Trichloroacetic Acid (TCA) Coexposures: Changes in Methylation Status*

In Section E.3.4.2.2, information on the effects of TCE and its metabolites was presented in regard to effects on methylation status. After 7 days of gavage dosing, TCE, TCA and DCA were reported to increased hypomethylation of the promoter regions of c-Jun and c-Myc genes in mouse whole liver DNA, however, Caldwell and Keshava (2006) concluded that hypomethylation did not appear to be a chemical-specific effect at the concentration used. Bull et al. (2004) suggested that hypomethylation occurs at higher exposure levels than those that induce liver tumors for TCE and its metabolites. Along with studies of methylation changes induced by a exposure to a single agent a Pereira et al. (2001) have attempted to examine the effects on methylation changes from coexposures. This study was also reviewed by Caldwell et al. (2008b).

Pereira et al. (2001) hypothesized that changes in the methylation status of DNA can be a key event for MOA for DCA- and TCA-induced liver carcinogenicity through changes in gene regulation, and that chloroform ($CHCl_3$) coexposure may result in modification of DNA methylation. As discussed in Caldwell et al. (2008b),

> After 17 days of exposure of exposure to $CHCl_3$ (0, 400, 800, 1,600 mg/L, $n = 6$ mice per treatment group) female B6C3F1 mice were coexposed to DCA or TCA (500 mg/kg) during the last 5 days of exposure to chloroform. As noted by Caldwell et al. (2007b), Pereira et al. (2001) reported the effects of hypomethylation of the promoter region of *c-Myc* gene and on expression of its mRNA in the whole livers of female B6C3F1 mice and thus, these results represent composite changes in DNA methylation status from a variety of cell types and for hepatocytes lumped from differing parts of the liver lobule. When given alone, DCA, TCA, and to a lesser extent, the highest concentration of $CHCl_3$ (1,600 mg/L), was reported to decrease methylation of the *c-myc* promoter region. Coadministration of $CHCl_3$ (at 800 and 1,600 mg/L) was reported to decrease DCA-induced hypomethylation while exposure to $CHCl_3$ had no effect on TCA-induced hypomethylation. Treatment with DCA, TCA, and, to a lesser extent $CHCl_{3,}$ was reported to increase levels of *c-myc* mRNA. While expression of *c-myc* mRNA was increased by DCA or TCA treatment, increasing coexposures to $CHCl_3$ were reported to attenuate the actions of DCA but not TCA. Thus, differences in methylation status and expression of the *c-myc* gene induced by DCA or TCA exposure was reported to be differentially modulated by coexposure to $CHCl_{3.}$ The authors suggest these differences support differing actions by DCA and TCA. However, whether these changes represent key events

in the induction of liver cancer is a matter of debate, especially as a "snapshot in time" approach for such a nonspecific endpoint.

In a coexposure study in which an "initiating agent" was used as a coexposure along with other coexposure, Pereira et al. (2001) treated male and female 15-day old B6C3F1 mice with MNU (a cause of liver and kidney tumors) and then, starting at 5 weeks of age, treated them with DCA (3.2 g/L) or TCA (4.0 g/L) along with coexposure to $CHCl_3$ (0, 800, or 1,600 mg/L) for 36 weeks. Mice were reported to be examined for evidence of promotion of liver and kidney tumors. The numbers of animals in the exposure groups were highly variable, ranging from 25 (female initiated mice exposed to DCA) to 6 (female initiated mice exposed to DCA and 1,600 mg/L $CHCl_3$), thus, limiting the power of the study to ascertain treatment-related changes. However, unlike Bull et al. (2004), all liver tissues were examined with incidences of foci, adenomas, carcinomas, and both adenoma and carcinoma reported separately for treatment groups. Multiplicity for a combination of adenomas and carcinomas were reported as well as the tincture of foci and tumors.

Although as noted by Caldwell et al. (2008b):

> [T]he statistical power of the study to detect change was very low, an examination of the pattern of tumors induced by coexposure to MNU and TCE metabolites in female mice suggested that: (1) DCA exposure increased the incidence of adenomas but not carcinomas; (2) TCA increased incidence of carcinomas with little change in adenoma incidence; (3) coexposure to 800 and 1,600 mg/L of $CHCl_3$ decreased adenoma incidence by DCA treatment but not TCA; and (4) $CHCl_3$ coexposure decreased multiplicity of TCA-induced tumors and foci, but not for DCA. Caldwell et al. (2008) also note that this study suggests a gender-related effect on tumor induction from this study with; (1) adenoma incidences similar in male and female mice treated with DCA, but carcinoma incidence greater in males; (2) adenoma and carcinoma incidences greater in males than females treated with TCA; (3) tumor multiplicity similar in both genders for DCA treatments, but lower in females mice for TCA; and (4) less of an inhibitory effect by $CHCl_3$ on adenoma incidence from DCA exposure in male mice.

Pereira et al. (2001) also described the tinctural characteristics of the specific lesions induced by their coexposure treatments. Both foci and tumors induced by DCA exposure in "initiated" mice were reported to be over 95% eosinophilic in females, while in males, 89% of the foci were eosinophilic and 91% of tumors were basophilic. Thus, not only was there a gender-related difference in the incidences of tumors and foci but also foci and tumor phenotype. $CHCl_3$ coexposure was reported to change the DCA-induced foci from primarily eosinophilic to basophilic (i.e., 11 vs. 75% basophilic) in male mice coexposed to MNU. In male and female mice, TCA-induced tumors and foci were basophilic with no effect of $CHCl_3$ on phenotype in MNU treated mice.

E.4.3.3. *Coexposures to Brominated Haloacetates: Implications for Common Modes of Action (MOAs) and Background Additivity to Toxicity*

As noted by Caldwell et al. (2008b), along with chlorinated haloacetates and other solvents, "coexposures with TCE and brominated haloacetates may occur through drinking water. These compounds may affect TCE toxicity in a similar fashion to their chlorinated counterparts. As bromide concentrations increase, brominated haloacetates increase in the water supply."

Kato-Weinstein et al. (2001) administered dibromoacetate (DBA), bromochloroacetate (BCA), bromodichloroacetate (BDCA), TCA, and DCA in drinking water at concentrations of 0.2−3 g/L for 12 weeks to B6C3F1 male mice. The focus of the study was to determine the similarity in action between the brominated and chlorinated haloacetates. Each of the haloacetates, given individually, were reported to increase liver/body weight ratios in a dose-dependent manner. The dihaloactates, DCA, BCA and DBA, caused liver glycogen accumulation both by chemical measurements in liver homogenates and in ethanol-fixed liver sections (to preserved glycogen) stained with PAS. For DCA, a maximal level of glycogen increase was observed at 4 weeks of exposure at a 2 g/L exposure concentration. They report a 1.60-fold of control percent liver/body weight and 1.50-fold of control glycogen content after 8 weeks of exposure to 2 g/L DCA in male B6C3F1 mice. The baseline level of glycogen content (~60 mg/g) and the increase in glycogen after DCA exposure was consistent with the results reported by Pereira et al. (2004). The percent liver/body weight data for control mice was for animals sacrifice at 20 weeks of age. The 4−12 week exposure to DCA were staggered so that all animals would be 20 weeks of age at sacrifice. Thus, the animals were at differing ages at the beginning of DCA treatments between the groups. However, as with Pereira et al. (2004) the ~10% increase in liver mass that the glycogen increases represent are lower than the total increase in liver mass reported for DCA exposure. The authors noted possible contamination of BCA with small percentages of DCA and DBA in their studies (i.e., 84% BCA, 6% DCA and 8% DBA). The trihaloacetates (TCA and low concentrations of BDCA) were reported to produce slight decreases in liver glycogen content, especially in the central lobular region in cells that tended to accumulate glycogen in control animals. These effects on liver glycogen were reported at the lowest dose examined (i.e., 0.3 g/L). At the highest concentration, BDCA was reported to induce a pattern of glycogen distribution similar to that of DCA in mice.

All dihaloacetates were reported to reduce serum insulin levels at high concentrations. Conversely, trihaloacetates were reported to have no significant effects on serum insulin levels. For the study of peroxisome proliferation and DNA synthesis, mice were treated to BCA, DBA, and BDCA for 2, 4, or 26 weeks. The effects on DNA synthesis were small for all brominated haloacetates with only DBA reported to show a significant increase in DNA synthesis at 2 and 4 weeks but not at 26 weeks (increase in DNA synthesis was 3-fold of the highest control level). Of note is the highly variable level of DNA synthesis reported for control values that varied to a much higher degree (~3−6-fold variation within control groups at the same time points) than did treatment-related changes. DBA was the only brominated haloacetate that was reported to consistently increased PCO activity as a percentage of control values (actual values and variability between controls were not reported) with a 2−3-fold increase in PCO activity at 0.3 to 3.0 g/L DBA. DBA-induced PCO activity increases were reported to be limited to 2−4 weeks of treatment in contrast to TCA, which the authors reported to increase PCO activity consistently over time.

Tao et al. (2004) reported DNA methylation, glycogen accumulation and peroxisome proliferation after exposure of female B6C3F1 mice and male Fischer 344 rats exposed to 1 or 2 g/L DBA in drinking water for 2 to 28 days. DBA was reported to induce dose-dependent DNA hypomethylation in whole mouse and rat liver after 7 days of exposure with suppression sustained for the 28-day exposure period. The expression of mRNA for *c-Myc* in mice and rats and mRNA expression of the *IGF-II* gene in female mice were reported to be increased during the same period. Both rats and mice were reported to exhibit increased glycogen with mice having increased levels at 2 day and rats at 4 days. DBA was reported to cause an increase in lauroyl-CoA oxidase activity (a marker of peroxisome proliferation) in both mice (after 7 days) and rats (after 4 days) that was sustained for 28 days. Methylation changes reported here for DBA exposure in rats and mice are consistent with those reported for TCA and DCA by Pereira et al. (2001) in mice. The pattern of glycogen accumulation was also similar to that reported for DCA by Kato-Weinstein et al. (2001) and suggests that the brominated analogues of TCE metabolites exhibited similar actions as their chlorinated counterparts. In regard to peroxisomal enzyme activities Kato-Weinstein et al. (2001) reported PCO activity to be limited to 2−4 weeks with Tao et al. (2004) reporting lauroyl-CoA oxidase activity to be sustained for the lengths of the study (28-days) for DBA.

As noted by Caldwell et al. (2008b), “given the similarity of DCA and DBA effects, it is plausible that DBA exposure also induces liver cancer. Melnick et al. (2007) reported the results of DBA exposure to F344/N rats and B6C3F1 mice exposed to DBA for 3 months or 2 years in drinking water (0, 0.05, 0.5, or 1.0 g/L DBA for 2 years). Neoplasms at multiple sites were reported in both species exposed to DBA for 2 years with no effects on survival and little effect on mean body weight in either species. Similar to TCE, DCA and TCA, the liver was reported to be a target of DBA exposure. After 3-months of exposure, there were dose-related increases in hepatocellular vacuolization and liver weight reported in rats and mice described as 'glycogen-like.'" The authors report that the major neoplastic effect of DBA in rats was induction of malignant mesotheliomas in males and increased incidence of mononuclear cell leukemia in males and females. For mice, the major neoplastic effect of DBA exposure was reported to be the increased incidence of hepatocellular adenomas and carcinomas at all exposure levels. In addition to these liver tumors, hepatoblastomas were also reported to be increased in all exposure groups of male mice and exceeded historical control rates. The incidence of alveolar/bronchiolar adenoma and carcinoma was reported to be increased in the 0.5 g/L group of male mice along with marginal increases in alveolar hyperplasia in DBA-treated groups. The authors reported that the increases in hepatocellular neoplasms were not associated with hepatocellular necrosis or regenerative hyperplasia and concluded that an early increase in hepatocyte proliferation were not likely involved in the MOA for DBA because no increases in hepatocyte DNA labeling index were observed in mice exposed for 26 days and the slight increase that occurred in male F344 rats was not accompanied by an increase in liver tumor response.

As noted by Caldwell et al. (2008b),

> [T]he results of Kato-Weinstein et al. (2001), Tao et al. (2004), and Melnick et al. (2007) are generally consistent for DBA and show a number of activities that may be common to TCE metabolites (i.e., brominated and chlorinated haloacetate analogues generally have similar effects on liver glycogen accumulation, serum insulin levels, peroxisome proliferation, hepatocyte DNA synthesis, DNA methylation status, and hepatocarcinogenicity). It is therefore, plausible that these effects may be additive in situations of coexposure. However, as noted by Melnick et al. (2007), methylation status, events associated with PPARα agonism, hepatocellular necrosis, and regenerative hyperplasia are not established as key events in the MOA of these agents, and the MOAs for DCA- and DBA-induced liver tumors are unknown.

E.4.3.4. *Coexposures to Ethanol: Common Targets and Modes of Action (MOAs)*

As noted in the U.S. EPA's draft TCE assessment (U.S. EPA, 2001), alcohol consumption is a common coexposure that has been noted to affect TCE toxicity with TCE exposure cited as potentially increasing the toxicity of methanol and ethanol, not only by altering their metabolism to aldehydes, but also by altering their detoxification (e.g., similar to the "alcohol flush" reported for those who have an inactive aldehyde dehydrogenase allele). As noted by Caldwell et al. (2008b) "chemical co-exposures from both the environment and behaviors such as alcohol consumption may have effects that overlap with TCE in terms of active agents, pharmacokinetics, pharmacodynamics, and/or target tissue toxicity." Caldwell et al. (2008b) also note:

> In their review of solvent risk (including TCE), Brautbar and Williams (2002) suggest that laboratory testing that is commonly used by clinicians to detect liver toxicity may not be sensitive enough to detect early liver hepatotoxicity from industrial solvents and that the final clinical assessment of hepatotoxicity and industrial solvents must take into account synergism with medications, drugs of use and abuse, alcohol, age-dependent toxicity, and nutrition. Although many of these factors may be important, the focus in this section is on the effects of ethanol. Contemporary literature reports effects similar to those of TCE's and previous reports indicate ethanol consumption impacts TCE toxicity in humans, affects the pharmacokinetics and toxicity of TCE in rats, and is also a risk factor for cancer.
>
> The association between malignant tumors of the upper gastrointestinal tract and liver and ethanol consumption is based largely on epidemiological evidence, and thought to be causally related [Bradford et al., 2005; Badger et al., 2003]. Studies of the mechanisms of ethanol carcinogenicity have suggested the importance of its metabolism, including induction of CYP2E1 associated increases in production of reactive oxygen species and enhanced activation of a variety of pro-carcinogens, alteration of retinol and retinoic acid metabolism, and the actions of the metabolite acetaldehyde [Badger et al., 2003]. While ethanol is primarily metabolized by alcohol dehydrogenase, it undergoes simultaneous oxidation to acetate by hepatic P450s, primarily CYP2E1. Both chronic ethanol consumption as well as TCE treatment induces CYP2E1 [U.S. EPA, 2001]. Oneta et al. (2002) report that even at moderate chronic ethanol consumption, hepatic CYP2E1 is induced in humans, which they suggest, may be of importance in the pathogenesis of alcoholic liver disease; of ethanol, drug, and vitamin A interactions; and in alcohol-associated carcinogenesis. Induction of CYP2E1 can cause oxidative stress to the liver from nicotinamide dinucleotide phosphate (NADPH)-dependent reduction of dioxygen to reactive products even in the absence of substrate, and subsequent apoptosis [Badger et al., 2003]. Bradford et al. (2005) suggest that CYP2E1, and not NADPH oxidase, is required for ethanol-induced oxidative DNA damage to rodent liver but that NADPH oxidase-derived oxidants are critical for the development of ethanol-induced liver injury.
>
> There is increasing evidence that acetaldehyde, which is toxic, mutagenic, and carcinogenic, rather than alcohol is responsible for its carcinogenicity [Badger et al., 2003]. Mitochondrial aldehyde dehydrogenase (ALDH2) disposes of acetaldehyde generated by the oxidation of ethanol, and ALDH2 inactivity

through mutation or polymorphism has been linked to esophageal cancer in humans (everyday drinkers and alcoholics) [Badger et al., 2003]. For instance, increased esophageal cancer risk was reported for patients with the ALDH3*1 polymorphism as well as increased acetaldehyde in their saliva. TCE exposure has also been reported to induce a similar alcohol flush in humans which may be linked to its ability to decrease ALDH activities at relatively low concentrations and thus conferring a similar status to individuals with inactive ALDH2 allele [Wang et al., 1999]. Whether the MOA for the buildup of acetaldehyde after ethanol and TCE co-exposure is: (1) the induction of CYP2E1 by TCE resulting in increased metabolism to acetaldehyde; (2) inhibition of ALDH and thus reduced clearance of acetaldehyde, or (3) a number of other actions are unknown. Crabb et al. (2001) reported 20−30% reductions in ALDH2 protein level by PPARα agonists (Clofibrate treatment in rats and WY treatment in both wild and PPARα-null mice). This could be another pathway for TCE-induced effects on ethanol metabolism. It is an intriguing possibility that the reported association between the increased risk of human esophageal cancer and TCE exposure [Scott and Chiu, 2006] could be related to TCE effects on mitochondrial ALDH, given a similar association of this endpoint with ethanol consumption or ALDH polymorphism.

Finally, ethanol ingestion may have significant effects on TCE pharmacokinetics. Baraona et al. (2002 a,b) reported that chronic, but not acute, ethanol administration increased the hepatotoxicity of peroxynitrite, a potent oxidant and nitrating agent, by enhancing concomitant production of nitric oxide and superoxide. They also reported that nitric oxide mediated the stimulatory effects of ethanol on blood flow. Ethanol markedly enhanced portal blood flow (2-fold increase), with no changes in the hepatic, splenic, or pancreatic arterial blood flows in rats.

E.4.3.5. *Coexposure Effects on Pharmacokinetics: Predictions Using Physiologically Based Pharmacokinetic (PBPK) Models*

Along with experimental evidence that has focused on chronic and acute experiments using rodents, the potential pharmacokinetic modulation of risk has also been recently published reports using PBPK models that may be useful in predicting coexposure effects. Caldwell et al. (2008b) also examined and discussed these approaches and note:

> An important issue for prediction of the effects and relationship on MOAs by co-exposure is the degree to which modulation of TCE toxicity by other agents can be quantified. Pharmacokinetics or the absorption, distribution, metabolism, and elimination of an agent, can be affected by internal and external co-exposure. Such information can help to identify the chemical species that may be causally associated with observed toxic responses, the MOA, and ultimately identify potentially sensitive subpopulations for an effect such as carcinogenicity.

Physiologically based pharmacokinetic (PBPK) models are often used to estimate and predict the toxicologically relevant dose of foreign compounds in the body and have been developed to predict effects on pharmacokinetics that are additive or less or greater than additive. One of the first such models was developed for TCE [Andersen et al., 1987]. Given that TCE, PERC, and methyl chloroform (MC) are often found together in contaminated groundwater, Dobrev et al. (2001) attempted to investigate the pharmacokinetic interactions among the three solvents to calculate defined "interaction thresholds" for effects on metabolism and expected toxicity. Their null hypothesis was defined as competitive metabolic inhibition being the predominant result for TCE given in combination with other solvents. Gas uptake inhalation studies were used to test different inhibition mechanisms. A PBPK model was developed using the gas uptake data to test multiple mechanisms of inhibitory interactions (i.e., competitive, noncompetitive, or uncompetitive) with the authors reporting competitive inhibition of TCE metabolism by MC and PERC in simulations of pharmacokinetics in the rat. Occupational exposures to chemical mixtures of the three solvents within their Threshold Limit Value (TLV)/TWA limits were predicted to result in a significant increase (22%) in TCE blood levels compared with single exposures.

Dobrev et al. (2002) extended this work to humans by developing an interactive human PBPK model to explore the general pharmacokinetic profile of two common biomarkers of exposure, peak TCE blood levels, and total amount of TCE metabolites generated in rats and humans. Increases in the TCE blood levels were predicted to lead to higher availability of the parent compound for GSH conjugation, a metabolic pathway that may be associated with kidney toxicity/carcinogenicity. A fractional change in TCE blood concentration of 15% for combined TLV exposures of the three chemicals (25/50/350 ppm of PERC/TCE/MC) resulted in a predicted 27% increase of the S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) metabolites, indicating a nonlinear risk increase due to combined exposures to TCE. Binary combinations of the solvents produced GST-mediated metabolite levels almost twice as high as the expected rates of increase in peak blood levels of the parent compound. The authors suggested that using parent compound peak blood levels (a less sensitive biomarker) would result in two to three times higher (i.e., less conservative) estimates of potentially safe exposure levels. In regard to the detection of metabolic inhibition by PERC and MC, the simulations showed TCE blood concentrations to be the more sensitive dose metric in rats, but the total of TCE metabolites to be the more sensitive dose measure in humans. Finally, interaction thresholds were predicted to be occurring at lower levels in humans than rats.

Thrall and Poet (2000) investigated the pharmacokinetic impact of low-dose co-exposures to toluene and TCE in male F344 rats *in vivo* using a real-time breath analysis system coupled with PBPK modeling. The authors report that, using the binary mixture to compare the measured exhaled breath levels from high- and low-dose exposures with the predicted levels under various metabolic interaction simulations (competitive, noncompetitive, or uncompetitive inhibition), the optimized competitive metabolic interaction description yielded an interaction parameter Ki value closest to the Michaelis-Menten affinity parameter (K_M) of the inhibitor solvent. This result suggested that competitive inhibition is the most plausible type of metabolic interaction between these two solvents.

Isaacs et al. (2004) have reported gas uptake co-exposure data for $CHCl_3$ and TCE. The question as to whether it is possible to use inhalation data in combination with PBPK modeling to distinguish between different metabolic interactions was addressed using sensitivity analysis theory. Recommendations were made for design of optimal experiments aimed at determining the type of inhibition mechanisms resulting from a binary co-exposure protocol. This paper also examined the dual nature of inhibition of each chemical in the pair to each other, which is that TCE and $CHCl_3$ were predicted to interact in a competitive manner. Even though as stated by Dobrev et al. (2001), other solvents inhibit TCE metabolism, it is also possible to quantify the synergistic interaction that TCE has on other solvents, using techniques such as gas uptake inhalation exposures.

Haddad et al. (2000) has developed a theoretical approach to predict the maximum impact that a mixture consisting of co-exposure to dichloromethane, benzene, TCE, toluene, PERC, ethylbenzene, m-, p-, and o-xylene, and styrene would have on venous blood concentration due to metabolic interactions in Sprague-Dawley rats. Two sets of experimental co-exposures were conducted. The first study evaluated the change in venous blood concentration after a 4 hour constant inhalation exposure to the 10 chemical mixtures. This experiment was designed to examine metabolic inhibition for this complex mixture. The second study was designed to study the impact of possible enzyme induction by using the same inhalation co-exposure after a 3 day pretreatment with the same 10 chemical mixture. The resulting venous concentration measurements for TCE from the first study were consistent with metabolic inhibition theory. The 10-chemical mixture was the most complex co-exposure used in this study. The authors stated that as mixture complexity increased, the resulting parent compound concentration time courses changed less, an observation which is consistent with metabolic inhibition. For the pretreatment study, the authors found a systematic decrease in venous concentration (due to higher metabolic clearance) for all chemicals except PERC. Overall, these studies suggest a complex metabolic interaction between TCE and other solvents.

A PBPK model for TCE including all its metabolites and their interactions can be considered a mixtures model where all metabolites have a common starting point in the liver. An integrated approach taking into account TCE metabolites and their metabolic inhibition and interactions among each other is suggested in Chiu et al. (2006b).

E.5. POTENTIALLY SUSCEPTIBLE LIFE STAGES AND CONDITIONS THAT MAY ALTER RISK OF LIVER TOXICITY AND CANCER

As described in Sections E.1.2, E.3.2.2, E.3.2.6, E.4.2.1, E.4.2.2, E.4.2.3, and E.4.2.4, there are a number of conditions that are associated with increased risk of liver cancer and toxicity that include age, use of a number of prescription medications including fibrates and statins, disease state (e.g., diabetes, NALD, viral infections) and exposure to external environmental contaminants that have an affect on TCE toxicity and targets. Obviously epigenetic and genetic factors play a role in determining the risk to the individual. In terms of liver cancer, there is general consensus that despite the associations that have been made with etiological factors and the risk of liver cancer, the mechanism is still unknown. The MOA of TCE toxicity is also unknown but exposure to TCE and its metabolites have shown in rodent models to induce liver cancer and in a fashion that is not consistent with only a hypothesized MOA of PPARα receptor activation that is in need of revision. However, multiple TCE metabolites have been shown to also induce liver cancer with varying effects on the liver that have also been associated with early stages of neoplasia (glycogen storage) or other actions associated with risk of hepatocarcinogenicity. The growing epidemic of obesity has been suggested to increase the risk of liver cancer and may reasonably increase the target population for TCE effects on the liver.

Lifestyle factors such as ethanol ingestion have not only been shown to increase liver cancer risk in those who already have fatty liver, but also to increase the toxicity of TCE. However, as noted by Caldwell et al. (2008b), while there is evidence to suggest that TCE exposure may increase the risk of liver toxicity and cancer, there are not data to support a quantitative estimate of how coexposures may modulate that risk.

> These findings can also serve to alert the risk manager to the possibility that multiple internal and external exposures to TCE that may act via differing MOAs for the production of liver effects. This information suggests a possible lack of "zero" background exposures and can help identify potential susceptible populations.
>
> Background levels of haloacetates in drinking water may add to the cumulative exposure an individual receives via the metabolism of TCE. The brominated haloacetates apparently share some common effects and pathways with their chlorinated counterparts. Thus, concurrent exposure of TCE, its metabolites, and other haloacetates may pose an additive response as well as an additive dose. However, personal exposures are difficult to ascertain and the effects of such co-exposures on toxicity are hard to quantify. EPA's guidance on cumulative risk assessments directs "each office to take into account cumulative risk issues in scoping and planning major risk assessments and to consider a broader scope that

integrates multiple sources, effects, pathways, stressors, and populations for cumulative risk analyses in all cases for which relevant data are available" [U.S. EPA, 1997]. Widespread exposure to possible background levels of TCE metabolites or co-contaminants and other extrinsic factors have the potential to affect TCE toxicity. However, the available data for co-exposures on TCE toxicity appears inadequate for quantifying these effects, particularly at environmental levels of contamination and exposure. Thus, the risk manager and assessor are going to be limited by not having information regarding either (1) the type of exposure data necessary to assess the magnitude of co-exposures that may affect toxicity, or (2) the potential quantitative impacts of these co-exposures that would enable specific adjustments to risk. Nonetheless, the risk manager should be aware that qualitatively a case can be made that extrinsic factors may affect TCE toxicity.

E.6. UNCERTAINTY AND VARIABILITY

Along with general conclusions about the coherence of data that enable conclusions about effects on the liver shown through experimental studies of TCE, there have also been extensive discussions throughout this report regarding the specific limitations of experimental studies whose design was limited by small and varying groups of animals and variability in control responses as well as reporting deficiencies. Section E.3.2.5 has brought forward the uncertainty in the MOA for liver cancer in general. The consistency of different animal models with human HCC is described in Section E.3.3, with Section E.3.2.2 providing a discussion of the promise and limitations of emerging technologies to study the MOAs of liver can in general and for TCE specifically. Issues regarding the target cell for HCC and the complexities of studying the MOA for a heterogeneous disease are described in Sections E.3.2.4 and E.3.2.8, respectively. Finally, the uncertainty regarding key events in how activation of the PPARα receptor my lead to hepatocarcinogenesis and the problems with extrapolation of results using the common paradigm to study them (exposure to high levels of WY-14,643 in abbreviated bioassays in knockout mice) are outlined in Section E.3.5.1. As such uncertainties are identified future research can focus on resolving them.

E.7. REFERENCES

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APPENDIX F

TCE Noncancer Dose-Response Analyses

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APPENDIX F: TCE NONCANCER DOSE-RESPONSE ANALYSES

F.1. DATA SOURCES

Data sources are cited in the body of this report in the section describing dose-response analyses (see Chapter 5).

F.2. DOSIMETRY

This section describes some of the more detailed dosimetry calculations and adjustments used in Section 5.1.

F.2.1. Estimates of Trichlorethylene (TCE) in Air From Urinary Metabolite Data Using Ikeda et al. (1972)

F.2.1.1. *Results for Chia et al. (1996)*

Chia et al. (1996) demonstrated a dose-related effect on hyperzoospermia in male workers exposed to trichloroethylene (TCE), lumping subjects into four groups based on range of trichloroacetic acid (TCA) in urine (see Table F-1).

Table F-1. Dose-response data from Chia et al. (1996)

TCA, mg per g creatinine	No. of subjects	No. with hyperzoospermia
0.8 to <25	37	6
50 to <75	18	8
75 to <100	8	4
≥100 to 136.4	5	3

Minimum and maximum TCA levels are reported in the text of Chia et al. (1996), the other data, in their Table 5.

Data from Ikeda et al. (1972) were used to estimate the TCE exposure concentrations corresponding to the urinary TCA levels reported by Chia et al. (1996). Ikeda et al. (1972) studied 10 workshops, in each of which TCE vapor concentration was “relatively constant.” They measured atmospheric concentrations of TCE and concentrations in workers’ urine of total trichloro compounds (TTC), TCA, and creatinine, and demonstrated a linear relation between TTC/creatinine (mg/g) in urine and TCE in the work atmosphere. Their data are tabulated as geometric means (the last column was calculated by us, as described in Table F-2).

Table F-2. Data on TCE in air (ppm) and urinary metabolite concentrations in workers reported by Ikeda et al. (1972)

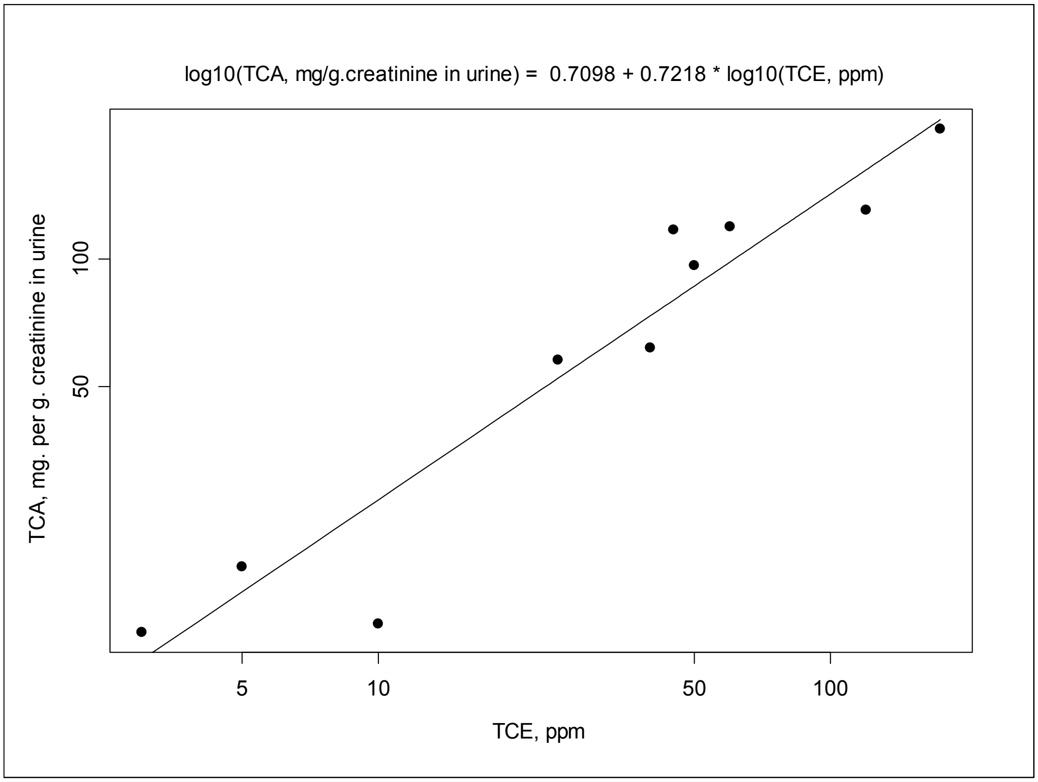
n	TCE (ppm)	TTC (mg/L)	TCA (mg/L)	TTC (mg/g creatinine)	TCA (mg/g creatinine)
9	3	39.4	12.7	40.8	13.15127
5	5	45.6	20.2	42.4	18.78246
6	10	60.5	17.6	47.3	13.76
4	25	164.3	77.2	122.9	57.74729
4	40	324.9	90.6	221.2	61.68273
5	45	399	138.4	337.7	117.137
5	50	418.9	146.6	275.8	96.52012
5	60	468	155.4	359	119.2064
4	120	915.3	230.1	518.9	130.4478
4	175	1210.9	235.8	1040.1	202.5399

These data were used to construct the last column "TCA.cr.mg.g" (mg TCA/g creatinine), as follows: TCA (mg/g creatinine) = TCA (mg/L) × TTC (mg/g creatinine)/TTC (mg/L). The regression relation between TCE (ppm) and TCA (mg/g creatinine) was evaluated using these data. Ikeda et al. (1972) reported that the measured values are lognormally distributed and exhibit heterogeneity of variance, and that the reported data (above) are geometric means. Thus, the regression relation between log10(TCA [mg/g creatinine]) and log10(TCE [ppm]) was used, assuming constant variances and using number of subjects "*n*" as weights. Figure F-1 shows the results.

Next, a Berkson setting for linear calibration was assumed, in which one wants to predict *X* (TCE, ppm) from means for *Y* (TCA, mg/g creatinine), with substantial error in *Y* (Snedecor and Cochran, 1980). Thus, the inverse prediction for the data of Chia et al. (1996) was used to infer their mean TCE exposures. The relation based on data from Ikeda et al. (1972) is

$$\log10(\text{TCA, mg/g creatinine}) = 0.7098 + 0.7218*\log10(\text{TCE, ppm}) \qquad \text{(Eq. F-1)}$$

and the inverse prediction is




```
Coefficients:
                Value Std. Error t value Pr(>|t|)
   (Intercept) 0.7098 0.1132     6.2688  0.0002
log10(TCE.ppm) 0.7218 0.0771     9.3578  0.0000

Residual standard error: 0.3206 on 8 degrees of freedom
Multiple R-Squared: 0.9163
F-statistic: 87.57 on 1 and 8 degrees of freedom, the p-value
is 0.0000139
```

Figure F-1. Regression of TCE in air (ppm) and TCA in urine (mg/g creatinine) based on data from Ikeda et al. (1972).

$$\log10(TCE) = [\log10(TCA) - 0.7098]/0.7218 \quad \text{(Eq. F-2)}$$

$$TCE, ppm = 10\^{}([\log10(TCA) - 0.7098]/0.7218)$$

Because of the lognormality of data reported by Ikeda et al. (1972), the means of the logarithms of the ranges for TCA (mg/g creatinine) in Chia et al. (1996), which are estimates of the median for the group, were used. The results are shown in Table F-3.

Table F-3. Estimated urinary metabolite and TCE air concentrations in dose groups from Chia et al. (1996)

TCA, mg per g Creatinine	Estim. TCA median[a]	Log10(TCA median)	Estim. ppm TCE[b]
0.8 to <25	4.47	0.650515	0.827685
50 to <75	61.2	1.787016	31.074370
75 to <100	86.6	1.937531	50.226119
≥100 to 136.4	117	2.067407	76.008668

[a] 10^(mean[log10(TCA limits in first column)]).
[b] 10^([log10(TCA median)] − 0.7098)/0.7218.

Dose-response relations for the data of Chia et al. (1996) were modeled using both the estimated medians for TCA (mg/g creatinine) in urine and estimated TCE (ppm in air) as doses. The TCE-TCA-TTC relations are linear up to about 75 ppm TCE (Figure 1 of Ikeda et al. 1972), and certainly in the range of the benchmark dose (BMD). As noted below (see Section F.2.2), the occupational exposure levels are further adjusted to equivalent continuous exposure for deriving the point of departure (POD).

F.2.1.2. *Results for Mhiri et al. (2004)*

The lowest-observed-adverse-effect level (LOAEL) group for abnormal trigeminal nerve somatosensory evoked potential reported in Mhiri et al. (2004) had a urinary TCA concentration of 32.6 mg TCA/mg creatinine. Using Eq. F-2, above gives an occupational exposure level = 10^([log10(32.6) − 0.7098]/0.7218) = 12.97404 ppm. As noted below (see Section F.2.2), the occupational exposure levels are further adjusted to equivalent continuous exposure for deriving the POD.

F.2.2. Dose Adjustments to Applied Doses for Intermittent Exposure

The nominal applied dose was adjusted for exposure discontinuity (e.g., exposure for 5 days per week and 6 hours per day reduced the dose by the factor [5/7]*[6/24]). The physiologically based pharmacokinetic (PBPK) dose metrics took into account the daily and weekly discontinuity to produce an equivalent average dose for continuous exposure. No dose adjustments were made for duration of exposure or a less-than-lifetime study, as is typically done for cancer risk estimates, though in deriving the candidate reference values, an uncertainty factor for subchronic-to-chronic exposure was applied where appropriate.

For human occupational studies, inhalation exposures (air concentrations) were adjusted by the number of work (vs. nonwork) days and the amount of air intake during working hours as a fraction of the entire day (10 m^3 during work/20 m^3 for entire day). For the TCE ppm in air converted from urinary metabolite data using Ikeda et al. (1972), the work week was 6 days, so the adjustment for number of work days is 6/7.

F.2.3. Physiologically Based Pharmacokinetic (PBPK) Model-Based Internal Dose Metrics

PBPK modeling was used to estimate levels of dose metrics corresponding to different exposure scenarios in rodents and humans (see Section 3.5). The selection of dose metrics for specific organs and endpoints is discussed under Section 5.1.

The PBPK model requires an average body weight. For most of the studies, averages specific to each species, strain, and sex were used. Where these were not reported in the text of an article, data were obtained by digitizing the body weight graphics (Maltoni et al., 1986) or by finding the median of weekly averages from graphs (National Cancer Institute [NCI], 1976; National Toxicology Program [NTP], 1990, 1988). Where necessary, default adult body weights specific to the strain were used (U.S. EPA, 1994).

F.3. DOSE-RESPONSE MODELING PROCEDURES

Where adequate dose-response data were available, models were fitted with the BenchMark Dose Software (BMDS) (http://www.epa.gov/ncea/bmds) using the applicable applied doses or PBPK model-based dose metrics for each combination of study, species, strain, sex, endpoints, and benchmark response (BMR) under consideration.

F.3.1. Models for Dichotomous Response Data

F.3.1.1. *Quantal Models*

For dichotomous responses, the log-logistic, multistage, and Weibull models were fitted. These models adequately describe the dose-response relationship for the great majority of data sets, specifically in past TCE studies (Filipsson and Victorin, 2003). If the slope parameter of the log-logistic model was less than 1, indicating a supralinear dose-response shape, the model with the slope constrained to 1 was also fitted for comparison. For the multistage model, an order one less than the number of dose groups was used, in addition to the 2^{nd}-order multistage model if it differed from the preceding model, and the first-order ('linear') multistage model (which is identical to a Weibull model with power parameter equal to 1). The Weibull model with the power parameter unconstrained was also fitted t.

F.3.1.2. *Nested Dichotomous Models*

In addition, nested dichotomous models were used for developmental effects in rodent studies to account for possible litter effects, such maternal covariates or intralitter correlation. The available nested models in BMDS are the nested log-logistic model, the Rai-VanRyzin models, and the NCTR model. Candidates for litter-specific covariates (LSC) were identified from the studies and considered legitimate for analysis if they were not significantly dose-related (determined via regression, analysis of variance). The need for a LSC was indicated by a difference of at least 3 in the Akaike Information Criteria (AIC) for models with and without a LSC. The need to estimate intralitter correlations (IC) was determined by presence of a high correlation coefficient for at least one dose group and by AIC. The fits for nested models were also compared with the results from quantal models.

F.3.2. Models for Continuous Response Data

For continuous responses, the distinct models available in BMDS were fitted: power model (power parameter unconstrained and constrained to ≥1), polynomial model, and Hill model. Both constant variance and modeled variance models were fit; but constant variance models were used for model parsimony unless the *p*-value for the test of homogenous variance was <0.10, in which case the modeled variance models were considered. For the polynomial model, model order was selected as follows. A model of order 1 was fitted first. The next higher order model (up to order $n-1$) was accepted if AIC decreased more than 3 units and the *p*-value for the mean did not decrease.

F.3.3. Model Selection

After fitting these models to the data sets, the recommendations for model selection set out in U.S. Environmental Protection Agency (U.S. EPA)'s *Benchmark Dose Technical Guidance Document* (Inter-Agency Review Draft, U.S. EPA, 2008b) were applied. First, models were generally rejected if the *p*-value for goodness of fit was <0.10. In a few cases in which none of the models fit the data with $p > 0.10$, linear models were selected on the basis of an adequate visual fit overall. Second, models were rejected if they did not appear to adequately fit the low-dose region of the dose-response relationship, based on an examination of graphical displays of the data and scaled residuals. If the benchmark dose lower bound (BMDL) estimates from the remaining models were "sufficiently close" (a criterion of within 2-fold for "sufficiently close" was used), then the model with the lowest AIC was selected. The AIC is a measure of information loss from a dose-response model that can be used to compare a set of models. Among a specified set of models, the model with the lowest AIC is considered the "best." If two or more models share the lowest AIC, the BMD *Technical Guidance Document* (U.S. EPA, 2008b) suggests that an average of the BMDLs could be used, but averaging was not used in this assessment (for the one occasion in which models shared the lowest AIC, a selection was made based on visual fit). If the BMDL estimates from the remaining models are not sufficiently close, some model dependence is assumed. With no clear biological or statistical basis to choose among them, the lowest BMDL was chosen as a reasonable conservative estimate, as suggested in the *Benchmark Dose Technical Guidance Document*, unless the lowest BMDL appeared to be an outlier, in which case further judgments were made.

F.3.4. Additional Adjustments for Selected Data Sets

In a few cases, the dose-response data necessitated further adjustments in order to improve model fits.

The behavioral/neurological endpoint “number of rears” from Moser et al. (1995) consisted of counts, measured at five doses and four measurement times (with eight observations each). The high dose for this endpoint was dropped because the mean was zero, and no monotone model could fit that well. Analysis of means and standard deviations for these counts suggested a Box-Cox power transform (Box et al., 1978) of ½ (i.e., square root) to stabilize variances (i.e., the slope of the regression of log[standard deviation (SD)] on log[mean] was 0.46, and the relation was linear and highly significant). This information was helpful in selecting a suitable variance model with high confidence (i.e., variance constant, for square-root transformed data). Thus, the square root was taken of the original individual count data, and the mean and variance of the transformed count data were used in the BMD modeling.

The high-dose group was dropped due to supra-linear dose-response shapes in two cases: fetal cardiac malformations from Johnson et al. (2003) and decreased PFC response from Woolhiser et al. (2006). Johnson et al. (2003) is discussed in more detail below (see Section F.4.2.1). For Woolhiser et al. (2006), model fit near the BMD and the lower doses as well as the model fit to the variance were improved by dropping the highest dose (a procedure suggested in U.S. EPA (2008b).

In some cases, the supralinear dose-response shape could not be accommodated by these measures, and a LOAEL or no-observed-adverse-effect level (NOAEL) was used instead. These include NCI (1976) (toxic nephrosis, >90% response at lowest dose), Keil et al. (2009) (autoimmune markers and decreased thymus weight, only two dose groups in addition to controls), and Peden-Adams et al. (2006) (developmental immunotoxicity, only two dose groups in addition to controls).

F.4. DOSE-RESPONSE MODELING RESULTS

F.4.1. Quantal Dichotomous and Continuous Modeling Results

The documents Appendix.linked.files\AppF.Non-cancer.Plots.TCE.contin.DRAFT.pdf and Appendix.linked.files\AppF.Non-cancer.Plots.TCE.dichot.DRAFT.pdf show the fitted model curves. The graphics include observations (group means or proportions), the estimated model curve (solid red line) and estimated BMD, with a BMDL. Vertical bars show 95% confidence intervals for the observed means. Printed above each plot are some key statistics (necessarily rounded) for model goodness of fit and estimated parameters. Printed in the plots in the upper left are the BMD and BMDL for the rodent data, in the same units as the rodent dose.

More detailed results, including alternative BMRs, alternative dose metrics, quantal analyses for endpoints for which nested analyses were performed, etc. are documented in the several spreadsheets. Input data for the analyses are in the following documents: Appendix.linked.files\AppF.Non-cancer.Input.Data.TCE.contin.DRAFT.pdf and Appendix.linked.files\AppF.Non-cancer.Input.Data.TCE.dichot.DRAFT.pdf. The documents Appendix.linked.files\AppF.Non-cancer.Results.TCE.contin.DRAFT.pdf and Appendix.linked.files\AppF.Non-cancer.Results.TCE.dichot.DRAFT.pdf present the data and model summary statistics, including goodness-of-fit measures (Chi-square goodness-of-fit *p*-value, AIC), parameter estimates, BMD, and BMDL. The group numbers "GRP" are arbitrary and are the same as GRP in the plots. Finally, note that not all plots are shown in the documents above, since these spreadsheets include many "alternative" analyses.

F.4.2. Nested Dichotomous Modeling Results

F.4.2.1. *Johnson et al. (2003) Fetal Cardiac Defects*

F.4.2.1.1. *Results using applied dose.* The biological endpoint was frequency of rat fetuses having cardiac defects, as shown in Table F-4. Individual animal data were kindly provided by Dr. Johnson (personal communication from Paula Johnson, University of Arizona, to Susan Makris, U.S. EPA, 26 August 2009). Cochran-Armitage trend tests using number of fetuses and number of litters indicated significant increases in response with dose (with or without including the highest dose).

One suitable candidate for a LSC was available: female weight gain during pregnancy. Based on goodness of fit, this covariate did not contribute to better fit and was not used. Some ICs were significant and these parameters were included in the model.

Table F-4. Data on fetuses and litters with abnormal hearts from Johnson et al. (2003)

Dose group (mg/kg/d):	0	0.00045	0.048	0.218	129
Fetuses					
Number of pups:	606	144	110	181	105
Abnormal heart:	13	0	5	9	11
Litters					
Number of litters:	55	12	9	13	9
Abnormal heart:	9	0	4	5	6

With the high dose included, the chi-square goodness of fit was acceptable, but some residuals were large (1.5 to 2) for the control and two lower doses. Therefore, models were also fitted after dropping the highest dose. For these, goodness of fit was adequate, and scaled residuals were smaller for the low doses and control. Predicted expected response values were closer to observed when the high dose was dropped, as shown in Table F-5:

Table F-5. Comparison of observed and predicted numbers of fetuses with abnormal hearts from Johnson et al. (2003), with and without the high-dose group, using a nested model

Dose group (mg/kg/d):	Abnormal hearts (pups)				
	0	0.00045	0.048	0.218	129
Observed:	13	0	5	9	11
Predicted expected:					
With high dose	19.3	4.5	3.5	5.7	11
Without high dose	13.9	3.3	3.4	10	--

Accuracy in the low-dose range is especially important because the BMD is based upon the predicted responses at the control and the lower doses. Based on the foregoing measures of goodness of fit, the model based on dropping the high dose was used.

The nested log-logistic and Rai-VanRyzin models were fitted; these gave essentially the same predicted responses and POD. The former model was used as the basis for a POD; results are in Table F-6 and Figure F-2.

Table F-6. Results of nested log-logistic model for fetal cardiac anomalies from Johnson et al. (2003) without the high-dose group, on the basis of applied dose (mg/kg/d in drinking water)

Model	LSC?	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	246.877	NA (df = 0)	0.01	0.252433	0.03776
NLOG	Y	N	251.203	0.0112	0.01	0.238776	0.039285
NLOG	N	N	248.853	0.0098	0.01	0.057807	0.028977
NLOG	N	Y	243.815	0.0128	0.1	0.71114	0.227675
NLOG	N	Y	243.815	0.0128	0.05	0.336856	0.107846
NLOG*	**N**	**Y**	**243.815**	**0.0128**	**0.01**	**0.064649**	**0.020698**

* Indicates model selected (Rai-VanRyzin model fits are essentially the same).

NLOG = "nested log-logistic" model.
LSC analyzed was female weight gain during pregnancy.

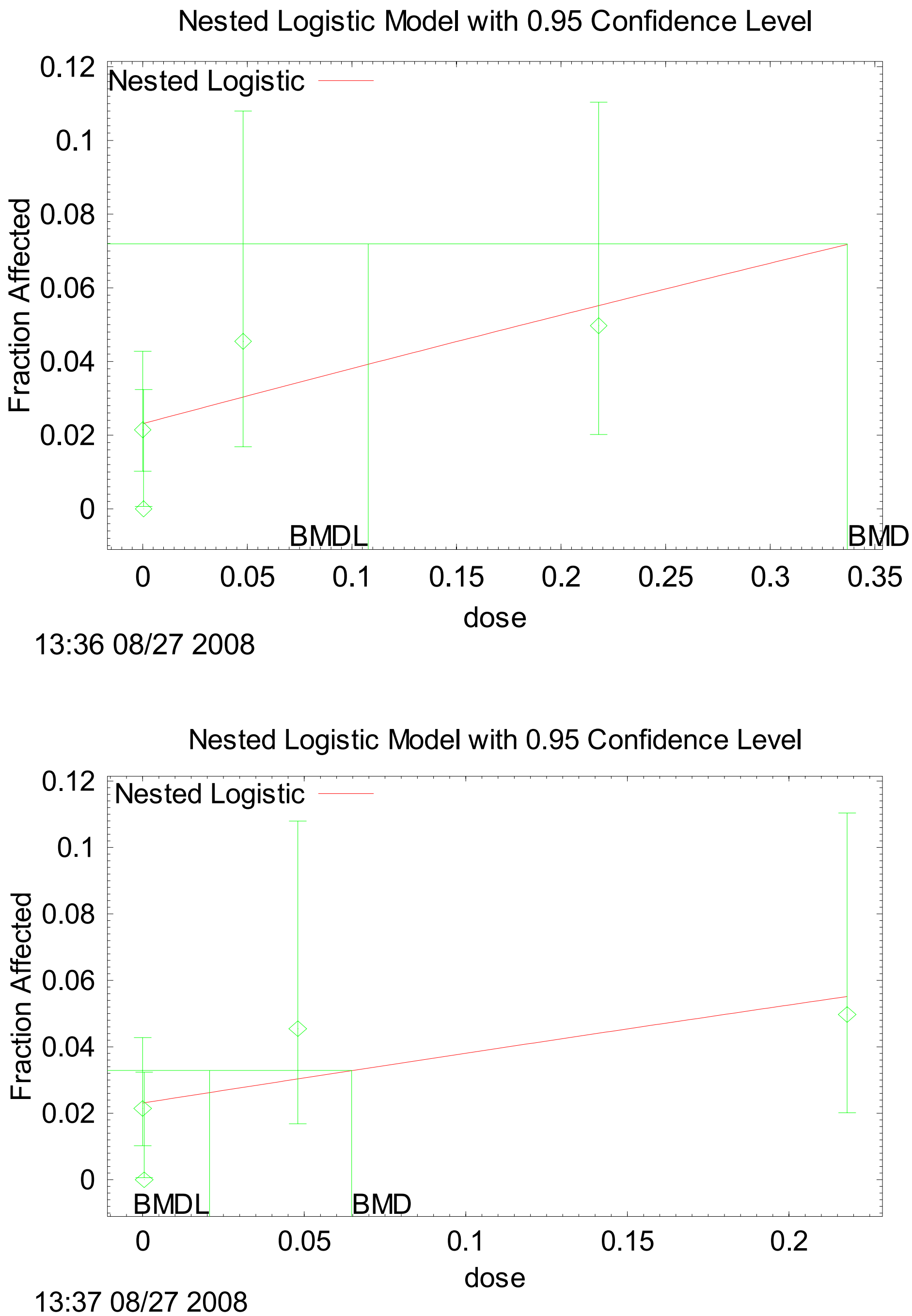


Figure F-2. BMD modeling of Johnson et al. (2003) using nested log-logistic model, with applied dose, without LSC, with IC, and without the high-dose group, using a BMR of 0.05 extra risk (top panel) or 0.01 extra risk (bottom panel).

F.4.2.1.2. *Chi-square Goodness of Fit Test for nested log-logistic.* The BMDS choice of subgroups did not seem appropriate given the data. The high-dose group of 13 litters was subdivided into three subgroups having sums of expected counts 3, 3, and 2. However, the control group of 55 litters could have been subdivided because expected response rates for controls were relatively high. There was also concern that the goodness of fit might change with alternative choices of subgroupings.

An R program was written to read the BMDS output, reading parameters and the table of litter-specific results (dose, covariate, estimated probability of response, litter size, expected response count, observed response count, scaled chi-square residual). The control group of 55 litters was subdivided into three subgroups of 18, 18, and 19 litters. Control litters were sampled randomly without replacement 100 times, each time creating 3 subgroups—i.e., 100 random assignments of the 55 control litters to three subgroups were made. For each of these, the goodness-of-fit calculation was made and the p-value saved. Within these 100 p-values, ≥75% were ≥0.05, and ≥50% had p-values ≥0.11, this indicated that the model is acceptable based on goodness-of-fit criteria.

F.4.2.1.3. *Results using physiologically based pharmacokinetic (PBPK) model-based dose metrics.* The nested log-logistic model was also run using the dose metrics in the dams of total oxidative metabolism scaled by body weight to the ¾-power (TotOxMetabBW34) and the area-under-the-curve of TCE in blood (AUCCBld). As with the applied dose modeling, LSC (maternal weight gain) was not included, but IC was included, based on the criteria outlined previously (see Section F.3.1.2). The results are summarized in Table F-7 and Figure F-3 for TotOxMetabBW34 and Table F-8 and Figure F-4 for AUCCBld.

F.4.2.2. *Narotsky et al. (1995)*

Data were combined for the high doses in the single-agent experiment and the lower doses in the 'five-cube' experiment. Individual animal data were kindly provided by Dr. Narotsky (personal communications from Michael Narotsky, U.S. EPA, to John Fox, U.S. EPA, 19 June 2008, and to Jennifer Jinot, U.S. EPA, 10 June 2008). Two endpoints were examined: frequency of eye defects in rat pups and prenatal loss (number of implantation sites minus number of live pups on postnatal day 1).

Table F-7. Results of nested log-logistic model for fetal cardiac anomalies from Johnson et al. (2003) without the high-dose group, using the TotOxMetabBW34 dose metric

Model	LSC?	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	246.877	NA (df = 0)	0.01	0.174253	0.0259884
NLOG	Y	N	251.203	0.0112	0.01	0.164902	0.0270378
NLOG	N	Y	243.815	0.0128	0.1	0.489442	0.156698
NLOG*	**N**	**Y**	**243.815**	**0.0128**	**0.01**	**0.0444948**	**0.0142453**
NLOG	N	N	248.853	0.0098	0.01	0.0397876	0.0199438

* Indicates model selected. BMDS failed with the Rai-VanRyzin and NCTR models.

NLOG = "nested log-logistic" model.
LSC analyzed was female weight gain during pregnancy.

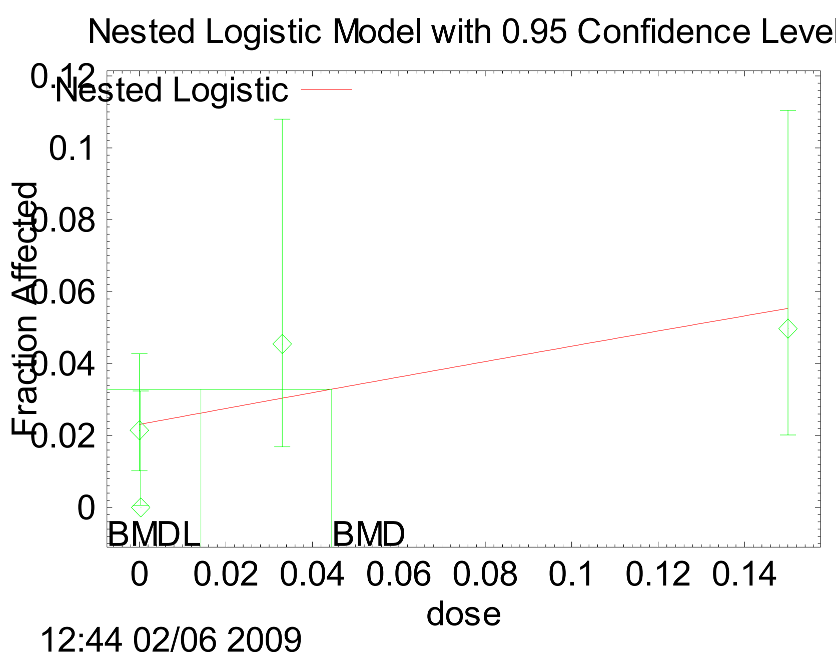


Figure F-3. BMD modeling of Johnson et al. (2003) using nested log-logistic model, with TotOxMetabBW34 dose metric, without LSC, with IC, and without the high-dose group, using a BMR of 0.01 extra risk.

Table F-8. Results of nested log-logistic model for fetal cardiac anomalies from Johnson et al. (2003) without the high-dose group, using the AUCCBld dose metric

Model	LSC?	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	246.877	NA (df = 0)	0.01	0.00793783	0.00118286
NLOG	Y	N	251.203	0.0112	0.01	0.00750874	0.00123047
NLOG*	N	Y	243.816	0.0128	0.1	0.0222789	0.00712997
NLOG*	**N**	**Y**	**243.816**	**0.0128**	**0.01**	**0.00202535**	**0.000648179**
NLOG	N	N	248.853	0.0098	0.01	0.00181058	0.000907513

* Indicates model selected. BMDS failed with the Rai-VanRyzin and NCTR models.

NLOG = "nested log-logistic" model.
LSC analyzed was female weight gain during pregnancy.

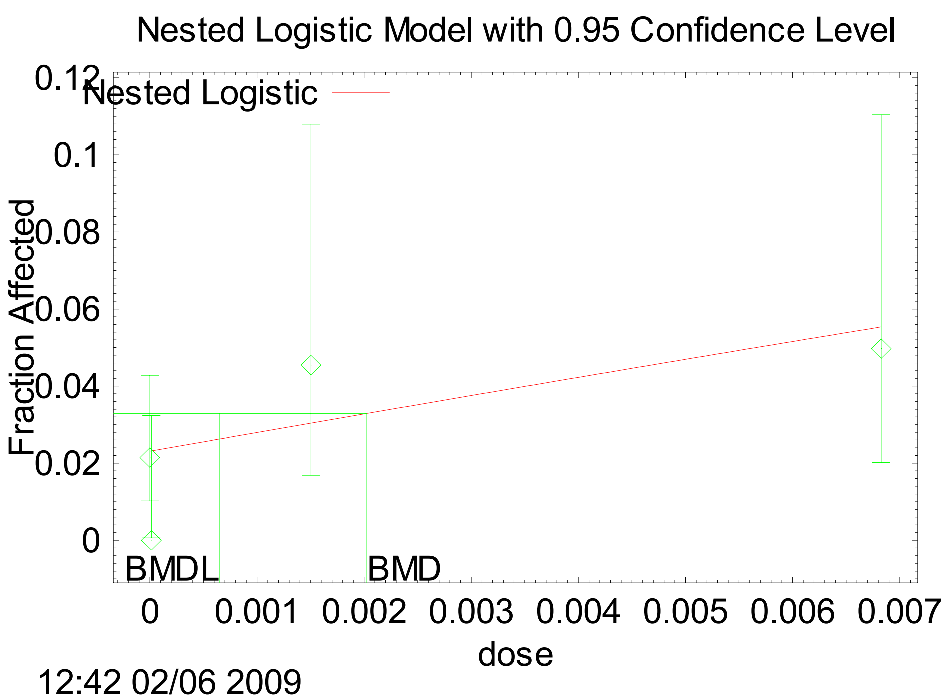


Figure F-4. BMD modeling of Johnson et al. (2003) using nested log-logistic model, with AUCCBld dose metric, without LSC, with IC, and without the high-dose group, using a BMR of 0.01 extra risk.

Two LSCs were considered, with analyses summarized in Table F-9. The number of implants is unrelated to dose, as inferred from regression and analysis of variance, and was considered as a LSC for eye defects. As number of implants is part of the definition for the endpoint of prenatal loss, it is not considered as a LSC for prenatal loss. A second LSC, the dam body weight on gestation day (GD) 6 (damBW6) was significantly related to dose and is unsuitable as a litter-specific covariate.

Table F-9. Analysis of LSCs with respect to dose from Narotsky et al. (1995)

Relation of litter-specific covariates to dose			
Implants:	none		
damBW6:	significant		
		Mean	Mean
	TCE	Implants	damBW6
	0	9.5	176.0
	10.1	10.1	180.9
	32	9.1	174.9
	101	7.8	170.1
	320	10.4	174.5
	475	9.7	182.4
	633	9.6	185.3
	844	8.9	182.9
	1,125	9.6	184.2
Using expt as covariate, e.g., damBW6 ~ TCE.mg.kgd + expt			
Linear regression		$p = 0.7486$	$p = 0.0069$
AoV (ordered factor)		$p = 0.1782$	$p = 0.0927$

Two LSCs were considered, with analyses summarized in Table F-9. The number of implants is unrelated to dose, as inferred from regression and analysis of variance, and was considered as a LSC for eye defects. As number of implants is part of the definition for the endpoint of prenatal loss, it is not considered as a LSC for prenatal loss. A second LSC, the dam body weight on GD 6 (damBW6) was significantly related to dose and is unsuitable as a litter-specific covariate.

F.4.2.2.1. ***Fetal eye defects.*** The nested log-logistic and Rai-VanRyzin models were fitted to the number of pups with eye defects reported by Narotsky et al. (1995), with the results summarized in Table F-10.

Table F-10. Results of nested log-logistic and Rai-VanRyzin model for fetal eye defects from Narotsky et al. (1995), on the basis of applied dose (mg/kg/d in drinking water)

Model	LSC?	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	255.771	0.3489	0.05	875.347	737.328[a]
NLOG	Y	N	259.024	0.0445	0.05	830.511	661.629
NLOG	N	Y	270.407	0.2281	0.05	622.342	206.460
NLOG	N	N	262.784	0.0529	0.10	691.93	542.101
NLOG	N	N	262.784	0.0529	0.05	427.389	264.386
NLOG	N	N	262.784	0.0529	0.01	147.41	38.7117[b]
RAI	Y	Y	274.339	0.1047	0.05	619.849	309.925
RAI	Y	N	264.899	0.0577	0.05	404.788	354.961
RAI	N	Y	270.339	0.2309	0.05	619.882	309.941
RAI	N	N	262.481	0.0619	0.10	693.04	346.52
RAI	N	N	262.481	0.0619	0.05	429.686	214.843
RAI	N	N	262.481	0.0619	0.01	145.563	130.938 [b]

[a] Graphical fit at the origin exceeds observed control and low dose responses and slope is quite flat (see Figure F-5), fitted curve does not represent the data well.
[b] Indicates model selected.

NLOG = "nested log-logistic" model; RAI = Rai-VanRyzin model.
LSC analyzed was implants.

Results for the nested log-logistic model suggested a better model fit with the inclusion of the LSC and IC, based on AIC. However, the graphical fit (see Figure F-5) is strongly sublinear and high at the origin where the fitted response exceeds the observed low-dose responses for the control group and two low-dose groups. An alternative nested log-logistic model without either LSC or IC (see Figure F-6), which fits the low-dose responses better, was selected. Given that this model had no LSC and no IC, the nested log-logistic model reduces to a quantal log-logistic model. Parameter estimates and the *p*-values were essentially the same for the two models (see

Table F-11). A similar model selection can be justified for the Rai-Van Ryzin model (see Figure F-7). Because no LSC and no IC were needed, this endpoint was modeled with quantal models, using totals of implants and losses for each dose group, which allowed choice from a wider range of models (those results appear with quantal model results in this appendix).

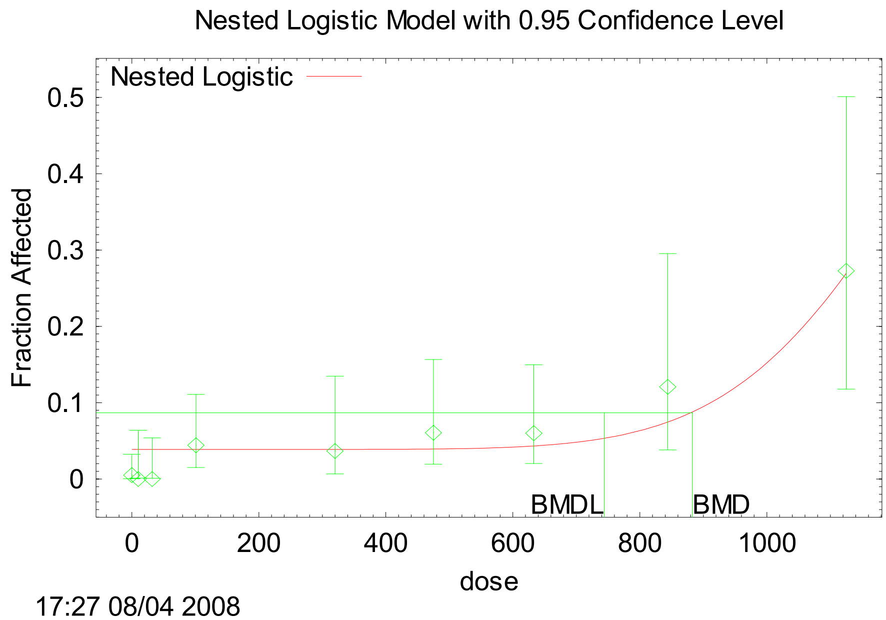


Figure F-5. BMD modeling of fetal eye defects from Narotsky et al. (1995) using nested log-logistic model, with applied dose, with both LSC and IC, using a BMR of 0.05 extra risk.

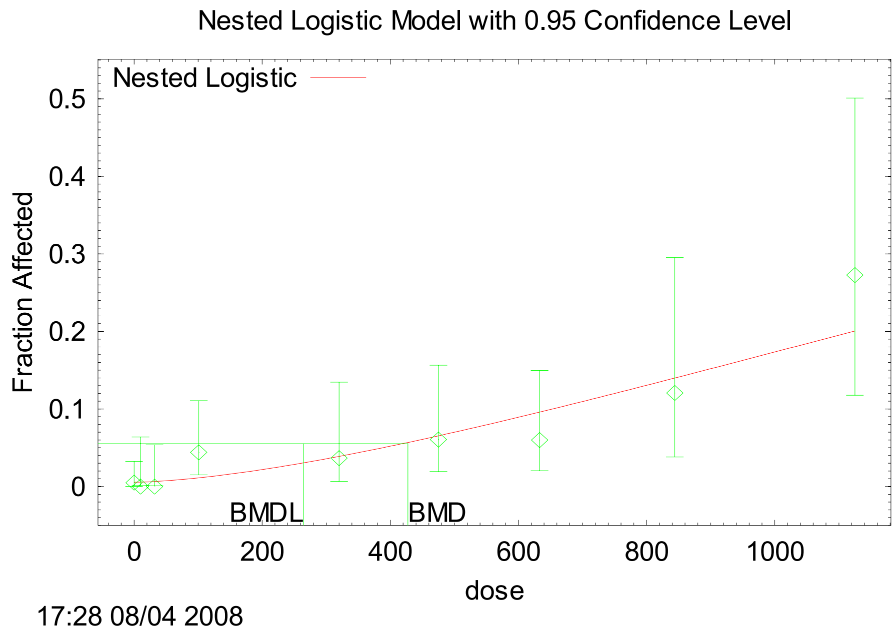


Figure F-6. BMD modeling of fetal eye defects from Narotsky et al. (1995) using nested log-logistic model, with applied dose, without either LSC or IC, using a BMR of 0.05 extra risk.

Table F-11. Comparison of results of nested log-logistic (without LSC or IC) and quantal log-logistic model for fetal eye defects from Narotsky et al. (1995)

Model	Parameter			BMD_{05}	$BMDL_{05}$
	Alpha	Beta	Rho		
Nested	0.00550062	-12.3392	1.55088	427.4	264.4
Quantal	0.00549976	-12.3386	1.55079	427.4	260.2

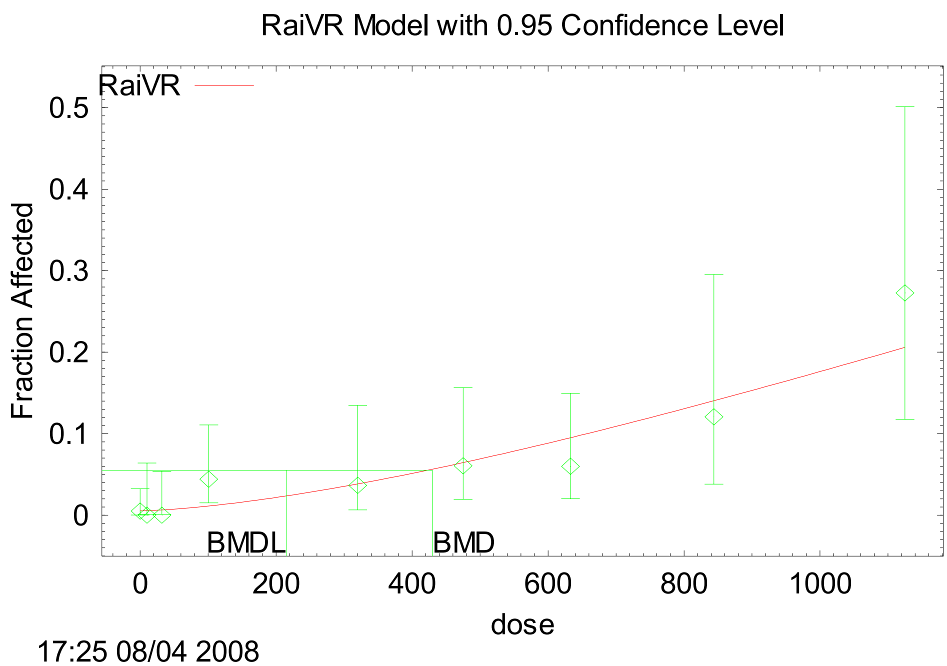


Figure F-7. BMD modeling of fetal eye defects from Narotsky et al. (1995) using nested Rai-VanRyzin model, with applied dose, without either LSC or IC, using a BMR of 0.05 extra risk.

F.4.2.2.2. ***Narotsky et al. (1995) prenatal loss.*** The nested log-logistic and Rai-VanRyzin models were fitted to prenatal loss reported by Narotsky et al. (1995), with the results summarized in Table F-12.

The BMDS nested models require a LSC, so dam body weight on GD6 ("damBW6") was used as the LSC. However, damBW6 is significantly related to dose and, so, is not a reliable LSC. Number of implants could not be used as a LSC because it was identified as number at risk in the BMDS models. These issues were obviated because the model selected did not employ the LSC.

Table F-12. Results of nested log-logistic and Rai-VanRyzin model for prenatal loss from Narotsky et al. (1995), on the basis of applied dose (mg/kg/d in drinking water)

Model	LSC?	IC?	AIC	Pval	BMR	BMD	BMDL
NLOG	Y	Y	494.489	0.2314	0.10	799.723	539.094
NLOG	Y	N	627.341	0.0000	0.10	790.96	694.673
NLOG	N	N	628.158	0.0000	0.10	812.92	725.928
NLOG	N	Y	490.766	0.2509	0.10	814.781	572.057
NLOG	N	Y	490.766	0.2509	0.05	738.749	447.077
NLOG	**N**	**Y**	**490.766**	**0.2509**	**0.01**	**594.995**	**252.437 ***
RAI	Y	Y	491.859	0.3044	0.10	802.871	669.059
RAI	Y	N	626.776	0.0000	0.10	819.972	683.31
RAI	N	N	626.456	0.0000	0.10	814.98	424.469
RAI	N	Y	488.856	0.2983	0.10	814.048	678.373
RAI	N	Y	488.856	0.2983	0.05	726.882	605.735
RAI	**N**	**Y**	**488.856**	**0.2983**	**0.01**	**562.455**	**468.713 ***

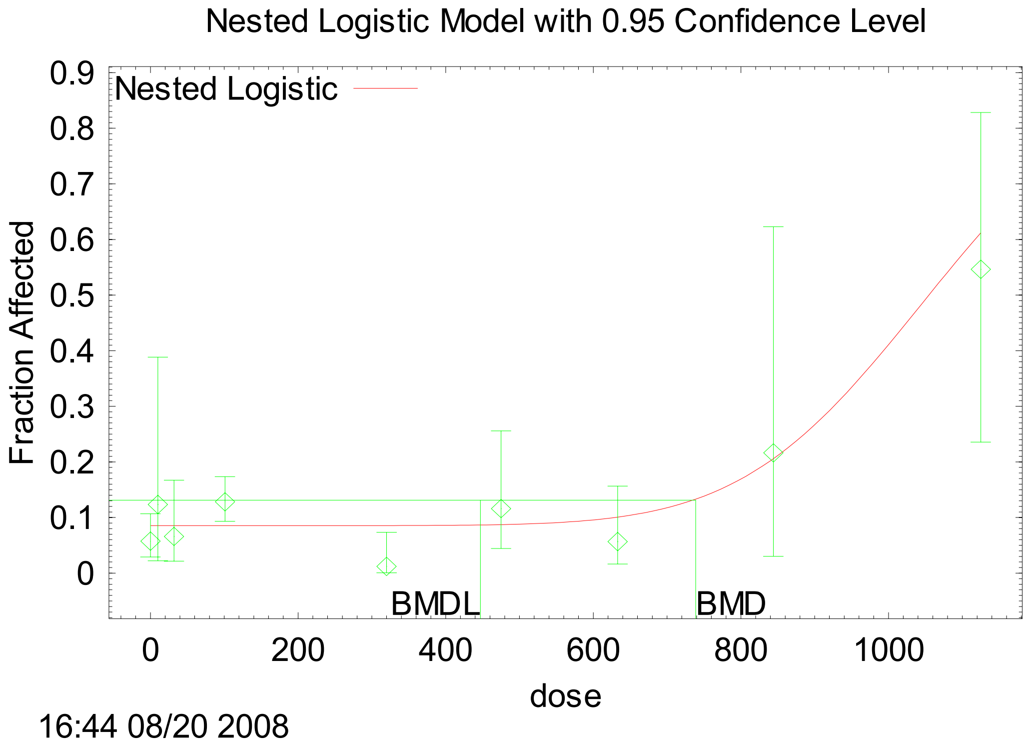
* Indicates model selected.

NLOG = "nested log-logistic" model; RAI = Rai-VanRyzin model.
LSC analyzed was dam body weight on GD6.

For the nested log-logistic models, the AIC is much larger when the IC is dropped, so the IC is needed in the model. The LSC can be dropped (and is also suspect because it is correlated with dose). The model with IC and without LSC was selected on the basis of AIC (shown in Figure F-8). For the Rai-VanRyzin models, the model selection was similar to that for the nested log-logistic, leading to a model with IC and without LSC, which had the lowest AIC (shown in Figure F-9).

F.4.3. Model Selections and Results

The final model selections and results for noncancer dose-response modeling are presented in Table F-13.



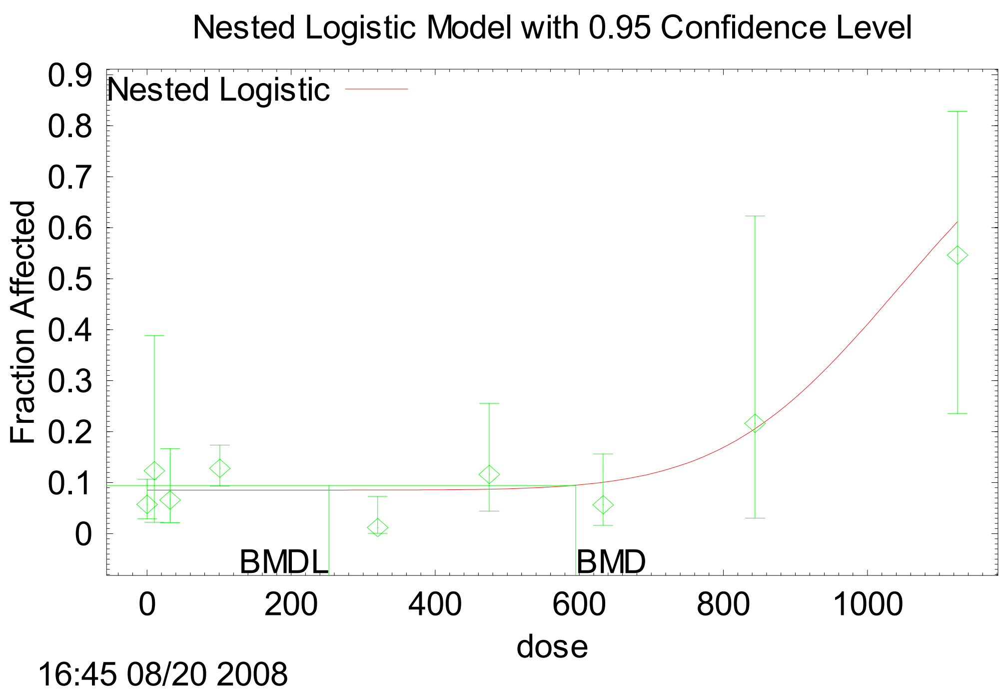
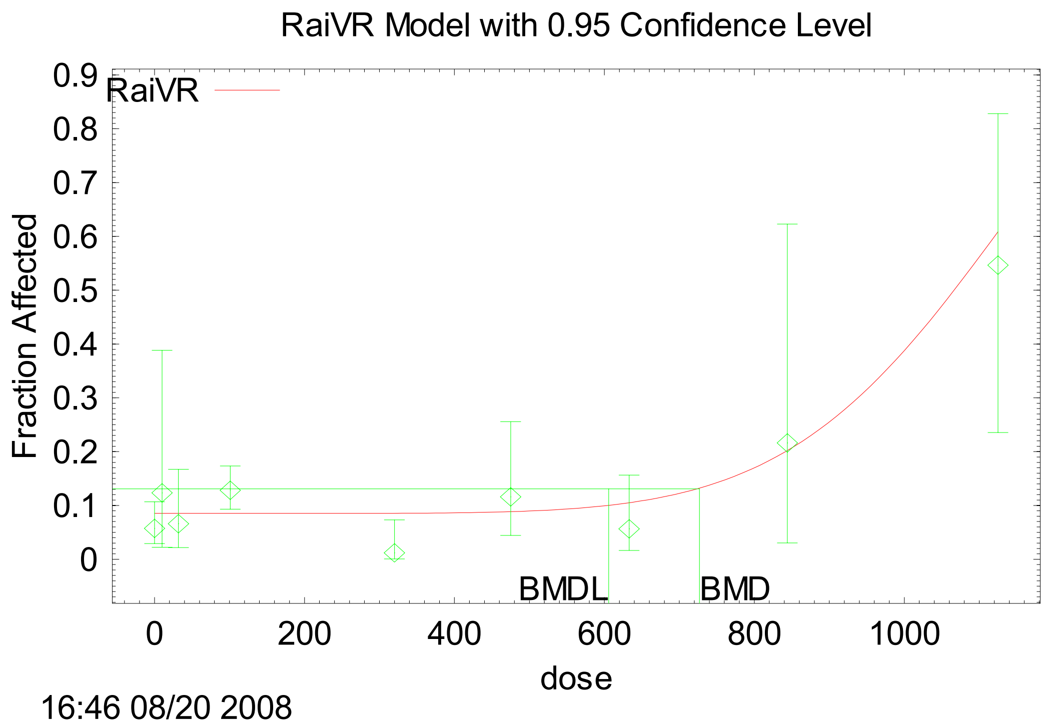


Figure F-8. BMD modeling of prenatal loss reported in Narotsky et al. (1995) using nested log-logistic model, with applied dose, without LSC, with IC, using a BMR of 0.05 extra risk (top panel) or 0.01 extra risk (bottom panel).



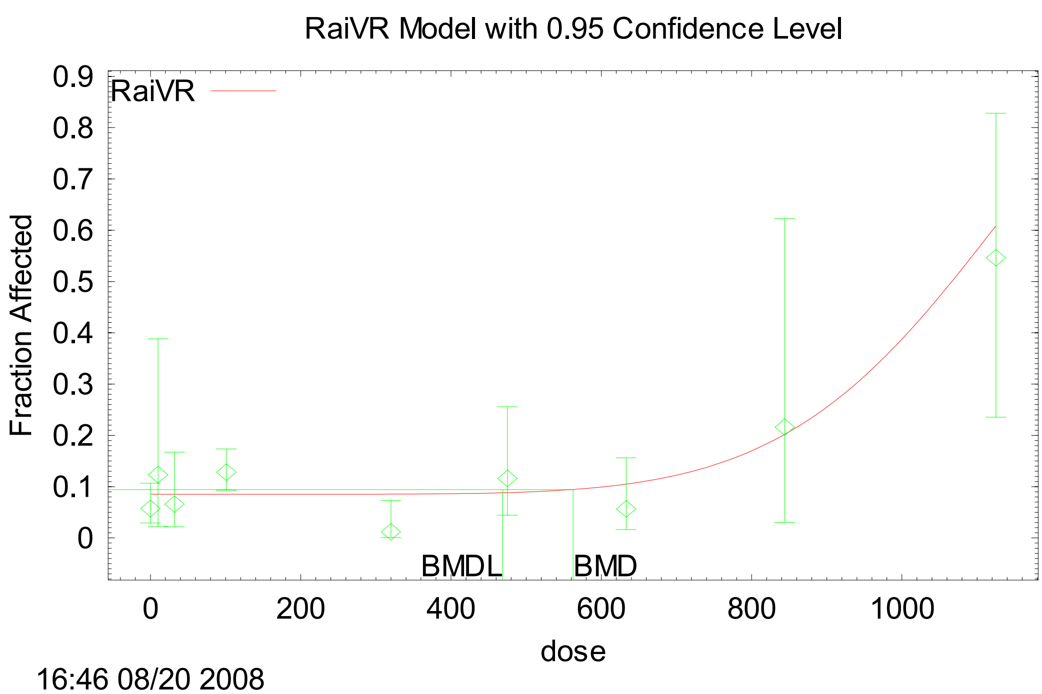


Figure F-9. BMD modeling of prenatal loss reported in Narotsky et al. (1995) using nested Rai-VanRyzin model, with applied dose, without LSC, with IC, using a BMR of 0.05 extra risk (top panel) or 0.01 extra risk (bottom panel).

Table F-13. Model selections and results for noncancer dose-response analyses

GRP	Study/run abbrev.	Species	Sex	Strain	Exp. route	Endpoint	Dose metric	BMR type	BMR	BMD/BMDL	BMDL	Model	Rep. BMD	Notes
Dichotomous models														
3	Chia et al., 1996	human	M	workers.elec.factory	inhal	N.hyperzoospermia	appl.dose	extra	0.1	2.14	1.43	loglogistic.1	3.06	
7	Narotsky et al., 1995	rat	F	F344	oral.gav	N.pups.eye.defects	appl.dose	extra	0.01	1.46	60.1	multistage	806	a
13	Narotsky et al., 1995.sa	rat	F	F344	oral.gav	N.dams.w.resorbed.litters	appl.dose	extra	0.01	5.47	32.2	multistage.2	570	
13	Narotsky et al., 1995.sa	rat	F	F344	oral.gav	N.dams.w.resorbed.litters	AUCCBld	extra	0.01	5.77	17.5	multistage.2	327	
13	Narotsky et al., 1995.sa	rat	F	F344	oral.gav	N.dams.w.resorbed.litters	TotMetabBW34	extra	0.01	1.77	77.5	weibull	156	
14	Johnson et al., 2003.drophi	rat	F	Sprague.Dawley	oral.dw	N.litters.abnormal.hearts	appl.dose	extra	0.1	2.78	0.0146	loglogistic.1	0.0406	b
36	Griffin et al., 2000	mice	F	MRL++	oral.dw	portal.infiltration	appl.dose	extra	0.1	2.67	13.4	loglogistic.1	35.8	
38	Maltoni et al., 1986	rat	M	Sprague.Dawley	inhal	megalonucleocytosis	appl.dose	extra	0.1	1.22	40.2	multistage	49.2	c
38	Maltoni et al., 1986	rat	M	Sprague.Dawley	inhal	megalonucleocytosis	ABioactDCVCBW34	extra	0.1	1.18	0.0888	loglogistic	0.105	
38	Maltoni et al., 1986	rat	M	Sprague.Dawley	inhal	megalonucleocytosis	AMetGSHBW34	extra	0.1	1.19	0.086	loglogistic	0.102	
38	Maltoni et al., 1986	rat	M	Sprague.Dawley	inhal	megalonucleocytosis	TotMetabBW34	extra	0.1	1.13	53.8	weibull	61	d
39	Maltoni et al., 1986	rat	M	Sprague.Dawley	oral.gav	megalonucleocytosis	appl.dose	extra	0.1	1.53	33.8	multistage.2	51.8	e
49	NTP, 1988	rat	F	Marshall	oral.gav	toxic nephropathy	appl.dose	extra	0.05	1.45	9.45	loglogistic.1	28.9	
49	NTP, 1988	rat	F	Marshall	oral.gav	toxic nephropathy	ABioactDCVCBW34	extra	0.05	1.45	0.0132	loglogistic.1	0.0404	
49	NTP, 1988	rat	F	Marshall	oral.gav	toxic nephropathy	AMetGSHBW34	extra	0.05	1.46	0.0129	loglogistic.1	0.0397	
49	NTP, 1988	rat	F	Marshall	oral.gav	toxic nephropathy	TotMetabBW34	extra	0.05	1.45	2.13	loglogistic.1	6.5	

Table F-13. Model selections and results for noncancer dose-response analyses (continued)

GRP	Study/run abbrev.	Species	Sex	Strain	Exp. route	Endpoint	Dose metric	BMR type	BMR	BMD/ BMDL	BMDL	Model	Rep. BMD	Notes
Nested dichotomous models														
NA	Johnson et al., 2003.drophi	rat	F	Sprague.Dawley	oral.dw	N.pups.abnormal.hearts	appl.dose	extra	0.01	3.12	0.0207	loglogistic.IC	0.711	b
NA	Johnson et al., 2003.drophi	rat	F	Sprague.Dawley	oral.dw	N.pups.abnormal.hearts	TotOxMetabBW34	extra	0.01	3.12	0.0142	loglogistic.IC		b
NA	Johnson et al., 2003.drophi	rat	F	Sprague.Dawley	oral.dw	N.pups.abnormal.hearts	AUCCBld	extra	0.01	3.12	0.000648	loglogistic.IC		b
NA	Narotsky et al., 1995	rat	F	F344	oral.gav	N.prenatal.loss	appl.dose	extra	0.01	1.2	469	RAI.IC	814	
Continuous models														
2	Land et al., 1981	mouse	M	(C57B1xC3H)F1	inhal	pct.abnormal.sperm	appl.dose	standard	0.5	1.33	46.9	polynomial.constvar	125	
6	Carney et al., 2006	rat	F	Sprague-Dawley (Crl:CD)	inhal	gm.wgt.gain.GD6.9	appl.dose	relative	0.1	2.5	10.5	hill	62.3	
8	Narotsky et al., 1995	rat	F	F344	oral.gav	gm.wgt.gain.GD6.20	appl.dose	relative	0.1	1.11	108	polynomial.constvar	312	
19	Crofton and Zhao. 1997	rat	M	Long-Evans	inhal	dB.auditory.threshold(16kHz)	appl.dose	absolute	10	1.11	274	polynomial.constvar	330	
21	George et al., 1986	rat	F	F344	oral.food	litters	appl.dose	standard	0.5	1.69	179	polynomial.constvar	604	
23	George et al., 1986	rat	F	F344	oral.food	live.pups	appl.dose	standard	0.5	1.55	152	polynomial.constvar	470	
26	George et al., 1986	rat	F	F344	oral.food	Foffspring.BWgm.day21	appl.dose	relative	0.05	1.41	79.7	polynomial.constvar	225	
34sq	Moser et al., 1995+persc om	rat	F	F344	oral.gav	no.rears	appl.dose	standard	1	1.64	248	polynomial.constvar	406	b,f
49	George et al., 1986	rat	F	F344	oral.food	traverse.time.21do	appl.dose	relative	1	1.98	72.6	power	84.9	
51	Buben and O'Flaherty, 1985	mouse	M	SwissCox	oral.gav	Liverwt.pctBW	appl.dose	relative	0.1	1.26	81.5	hill.constvar	92.8	

Table F-13. Model selections and results for noncancer dose-response analyses (continued)

GRP	Study/run abbrev.	Species	Sex	Strain	Exp. route	Endpoint	Dose metric	BMR type	BMR	BMD/ BMDL	BMDL	Model	Rep. BMD	Notes
51	Buben and O'Flaherty, 1985	mouse	M	SwissCox	oral.gav	Liverwt.pctBW	AMetLiv1BW34	relative	0.1	1.08	28.6	polynomial.constvar	28.4	
51	Buben and O'Flaherty, 1985	mouse	M	SwissCox	oral.gav	Liverwt.pctBW	TotOxMetabBW34	relative	0.1	1.08	37	polynomial.constvar	36.7	
58	Kjellstrand et al, 1983b	mouse	M	NMRI	inhal	Liverwt.pctBW	appl.dose	relative	0.1	1.36	21.6	hill	30.4	
58	Kjellstrand et al, 1983b	mouse	M	NMRI	inhal	Liverwt.pctBW	AMetLiv1BW34	relative	0.1	1.4	22.7	hill	32.9	
58	Kjellstrand et al, 1983b	mouse	M	NMRI	inhal	Liverwt.pctBW	TotOxMetabBW34	relative	0.1	1.3	73.4	hill	97.7	
60.Rp	Kjellstrand et al, 1983b	mouse	M	NMRI	inhal	Kidneywt.pctBW	appl.dose	relative	0.1	1.17	34.7	polynomial	47.1	
60.Rp	Kjellstrand et al, 1983b	mouse	M	NMRI	inhal	Kidneywt.pctBW	AMetGSHBW34	relative	0.1	1.18	0.17	polynomial	0.236	
60.Rp	Kjellstrand et al, 1983b	mouse	M	NMRI	inhal	Kidneywt.pctBW	TotMetabBW34	relative	0.1	1.17	71	polynomial	95.2	
63	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	Antibody.Forming Cells	appl.dose	standard	1	1.94	31.2	power.constvar	60.6	b
62	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	Antibody.Forming Cells	AUCCBld	standard	1	1.44	149	polynomial	214	
62	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	Antibody.Forming Cells	TotMetabBW34	standard	1	1.5	40.8	polynomial	61.3	
65	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	appl.dose	relative	0.1	4.29	15.7	hill.constvar	54.3	
65	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	ABioactDCVCBW34	relative	0.1	4.27	0.0309	hill.constvar	0.103	
65	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	AMetGSHBW34	relative	0.1	4.28	0.032	hill.constvar	0.107	
65	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	kidney.wt.per100gm	TotMetabBW34	relative	0.1	1.47	40.8	polynomial.constvar	52.3	
67	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	liver.wt.per100gm	appl.dose	relative	0.1	4.13	25.2	hill.constvar	70.3	
67	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	liver.wt.per100gm	AMetLiv1BW34	relative	0.1	1.53	46	polynomial.constvar	56.1	
67	Woolhiser et al, 2006	rat	F	CD (Sprague-Dawley)	inhal	liver.wt.per100gm	TotOxMetabBW34	relative	0.1	1.53	48.9	polynomial.constvar	59.8	

Table F-13. Model selections and results for noncancer dose-response analyses (continued)

[a]Eight-stage multistage model.
[b]Dropped highest dose.
[c]Three-stage multistage model.
[d]Weibull selected over log-logistic with the same AIC on basis of visual fit (less extreme curvature).
[e]Second-order MS selected on basis of visual fit (less extreme curvature).
[f]Square-root transformation of original individual count data.

Applied dose BMDLs are in units of ppm in air for inhalation exposures and mg/kg/d for oral exposures. Internal dose BMDLs are in dose metric units. Reporting BMD is BMD using a BMR of 0.1 extra risk for dichotomous models, and 1 control SD for continuous models.
Log-logistic = unconstrained log-logistic; log-logistic.1 = constrained log-logistic; multistage = multistage with #stages=dose groups-1; multistage.n = n-stage multistage; log-logistic.IC = nested log-logistic with IC, without LSC; RAI.IC = Rai-VanRyzin model with IC, without LSC; zzz.constvar = continuous model zzz with constant variance (otherwise variance is modeled).
Rep. = reporting, Exp. = exposure, Abbrev. = abbreviation.

F.5. DERIVATION OF POINTS OF DEPARTURE

F.5.1. Applied Dose Points of Departure

For oral studies in rodents, the POD on the basis of applied dose in mg/kg/d was taken to be the BMDL, NOAEL, or LOAEL. NOAELs and LOAELs were adjusted for intermittent exposure to their equivalent continuous average daily exposure (for BMDLs, the adjustments were already performed prior to BMD modeling).

For inhalation studies in rodents, the POD on the basis of applied dose in ppm was taken to be the BMDL, NOAEL, or LOAEL. NOAELs and LOAELs were adjusted for intermittent exposure to their equivalent continuous average daily exposure (for BMDLs, the adjustments were already performed prior to BMD modeling). These adjusted concentrations are considered human equivalent concentrations, in accordance with U.S. EPA (1994), as TCE is considered a Category 3 gas (systemically acting) and has a blood-air partition coefficient in rodents greater than that in humans (see Section 3.1).

F.5.2. Physiologically Based Pharmacokinetic (PBPK) Model-Based Human Points of Departure

As discussed in Section 5.1.3, the PBPK model was used for simultaneous interspecies (for endpoints in rodent studies), intraspecies, and route-to-route extrapolation based on the estimates from the PBPK model of the internal dose points of departure (idPOD) for each candidate critical study/endpoints. The following documents contain figures showing the derivation of the human equivalent doses and concentrations (human equivalent doses [HEDs] and human equivalent concentrations [HECs]) for the median (50th percentile) and sensitive (99th percentile) individual from the (rodent or human) study idPOD. In each case, for a specific study/endpoint(s)/sex/species (in the figure main title), and for a particular dose metric (Y-axis label), the horizontal line shows the original study idPOD (a BMDL, NOAEL, or LOAEL as noted) and where it intersects with the human 99th percentile (open square) or median (closed square) exposure-internal-dose relationship:

Appendix.linked.files\AppF.Non-cancer.HECs.Plots.human.inhalation.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HECs.Plots.rodent.inhalation.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HECs.Plots.rodent.oral.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HEDs.Plots.human.inhalation.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HEDs.Plots.rodent.inhalation.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HEDs.Plots.rodent.oral.studies.TCE.DRAFT.pdf

The original study internal doses are based on the median estimates from about 2,000 “study groups” (for rodent studies) or “individuals” (for human studies), and corresponding exposures for the human median and 99th percentiles were derived from a distribution of 2,000 "individuals." In both cases, the distributions reflect combined uncertainty (in the population means and variances) and population variability.

In addition, as part of the uncertainty/variability analysis described in Section 5.1.4.2, the POD for studies/endpoints for which BMD modeling was done was replaced by the LOAEL or NOAEL. This was done to because there was no available tested software for performing BMD modeling in such a context and because of limitations in time and resources to develop such software. However, the relative degree of uncertainty/variability should be adequately captured in the use of the LOAEL or NOAEL. The graphical depiction of the HEC_{99} or HED_{99} using these alternative PODs is shown in the following files:
Appendix.linked.files\AppF.Non-cancer.HECs.AltPOD.Plots.rodent.inhalation.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HECs.AltPOD.Plots.rodent.oral.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HEDs.AltPOD.Plots.rodent.inhalation.studies.TCE.DRAFT.pdf
Appendix.linked.files\AppF.Non-cancer.HEDs.AltPOD.Plots.rodent.oral.studies.TCE.DRAFT.pdf.

F.6. SUMMARY OF POINTS OF DEPARTURE (PODs) FOR CRITICAL STUDIES AND EFFECTS SUPPORTING THE INHALATION REFERENCE CONCENTRATION (RfC) AND ORAL REFERENCE DOSE (RfD)

This section summarizes the selection and/or derivation of PODs from the critical studies and effects that support the inhalation reference concentration (RfC) and oral reference dose (RfD). In particular, for each endpoint, the following are described the dosimetry (adjustments of continuous exposure, PBPK dose metrics), selection of BMR and BMD model (if BMD modeling was performed), and derivation of the human equivalent concentration or dose for a sensitive individual (if PBPK modeling was used). Section 5.1.3.1 discusses the dose metric selection for different endpoints.

F.6.1. National Toxicology Program (NTP, 1988)—Benchmark Dose (BMD) Modeling of Toxic Nephropathy in Rats

The critical endpoint here is toxic nephropathy in female Marshall rats (NTP, 1988), which was the most sensitive sex/strain in this study, although the differences among different sex/strain combinations was not large (BMDLs differed by ≤3-fold).

F.6.1.1. *Dosimetry and Benchmark Dose (BMD) Modeling*

Rats were exposed to 500 or 1,000 day, 5 days/week, for 104 weeks. The primary dose metric was selected to be average amount of dichlorovinyl cysteine (DCVC) bioactivated/kg$^{3/4}$/day, with median estimates from the PBPK model for the female Marshall rats in this study of 0.47 and 1.1.

Figure F-10 shows BMD modeling for the dichotomous models used (see Section F.5.1, above). The log-logistic model with slope constrained to ≥1 was selected because (1) the log-logistic model with unconstrained slope yielded a slope estimate <1 and (2) it had the lowest AIC.

The idPOD of 0.0132 mg DCVC bioactivated/kg$^{3/4}$/day was a BMDL for a BMR of 5% extra risk. This BMR was selected because toxic nephropathy is a clear toxic effect. This BMR required substantial extrapolation below the observed responses (about 60%); however, the response level seemed warranted for this type of effect and the ratio of the BMD to the BMDL was not large (1.56 for the selected model).

F.6.1.2. *Derivation of HEC_{99} and HED_{99}*

The HEC_{99} and HED_{99} are the lower 99th percentiles for the continuous human exposure concentration and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.0056 ppm and HED_{99} of 0.00338 mg/kg/d for the 99th percentile for uncertainty and variability are shown in Figure F-11. These values are used as this critical effect's POD to which additional uncertainty factors (UFs) are applied.

F.6.2. National Cancer Institute (NCI, 1976)—Lowest-Observed-Adverse-Effect Level (LOAEL) for Toxic Nephrosis in Mice

The critical endpoint here is toxic nephrosis in female B6C3F1 mice (NCI, 1976), which was the most sensitive sex in this study, although the LOAEL for males differed by less than 50%.

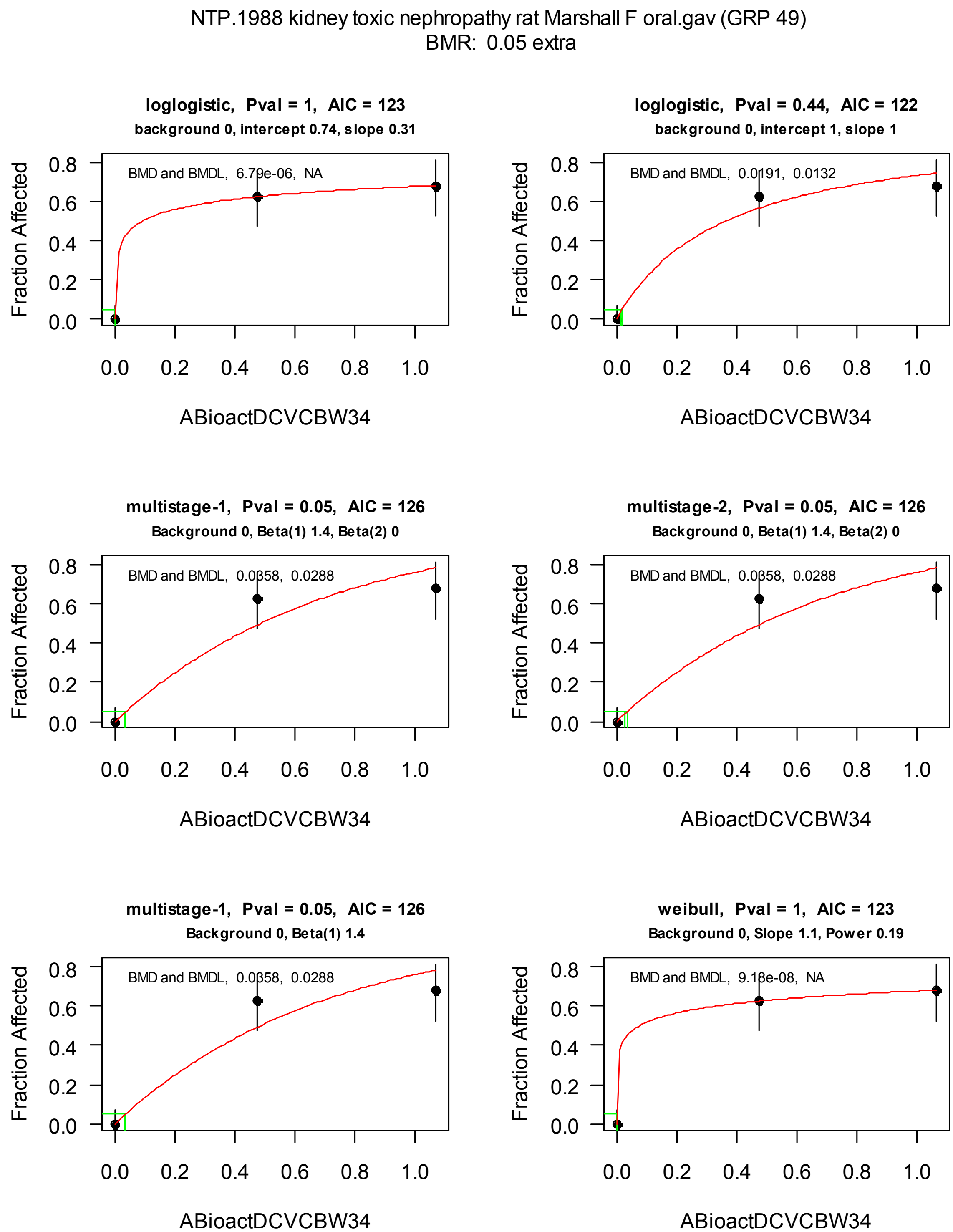


Figure F-10. BMD modeling of NTP (1988) toxic nephropathy in female Marshall rats.

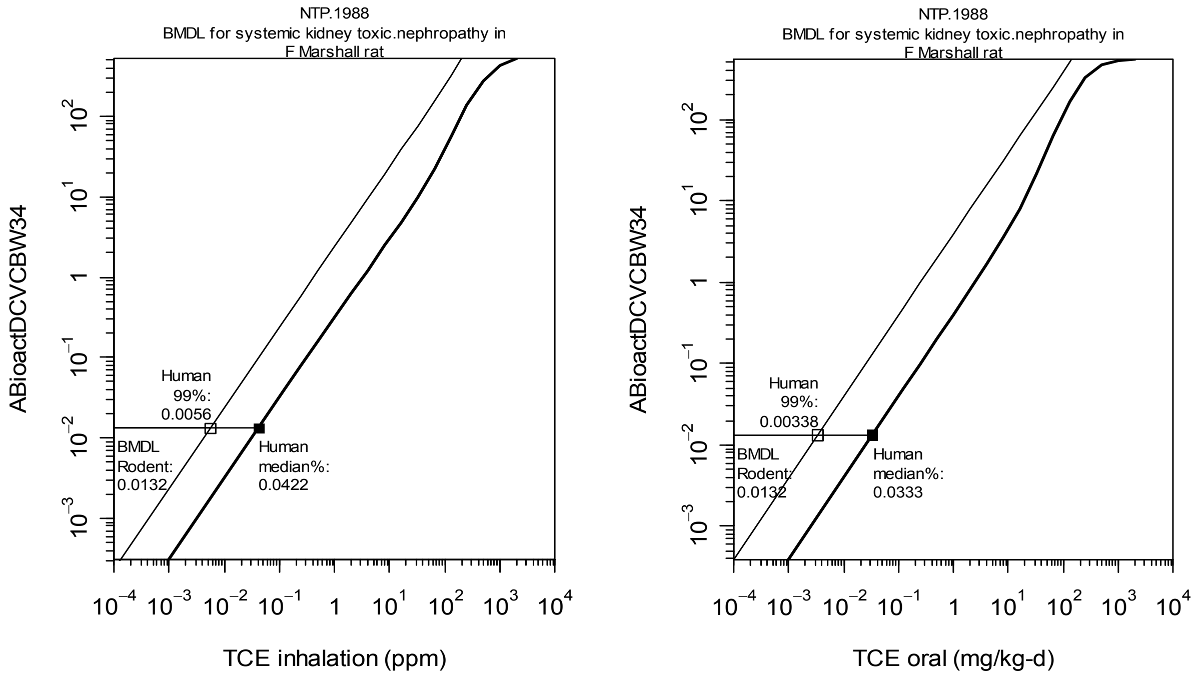


Figure F-11. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from NTP (1988) toxic nephropathy in rats.

F.6.2.1. *Dosimetry*

Mice were exposed to a time-weighted average of 869 and 1,739 mg/kg/d, 5 days/week, for 78 weeks. BMD modeling was not performed because the response at the LOAEL was >90%. The primary dose metric was selected to be average amount of TCE conjugated with glutathione (GSH)/$kg^{3/4}$/d. In this study, the lower dose group was exposed to two different dose levels (700 mg/kg/d for 12 weeks and 900 mg/kg/d for 66 weeks). The median estimates from the PBPK model for the two dose levels were 0.583 and 0.762 mg TCE conjugation with GSH/$kg^{3/4}$/d. Applying the same time-weighted averaging gives an idPOD LOAEL of 0.735 mg TCE conjugation with GSH/$kg^{3/4}$/d.

F.6.2.2. *Derivation of HEC_{99} and HED_{99}*

The HEC_{99} and HED_{99} are the lower 99^{th} percentiles for the continuous human exposure concentration and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.50 ppm and HED_{99} of 0.30 mg/kg/d for the

99th percentile for uncertainty and variability are shown in Figure F-12. These values are used as this critical effect's POD to which additional UFs are applied.

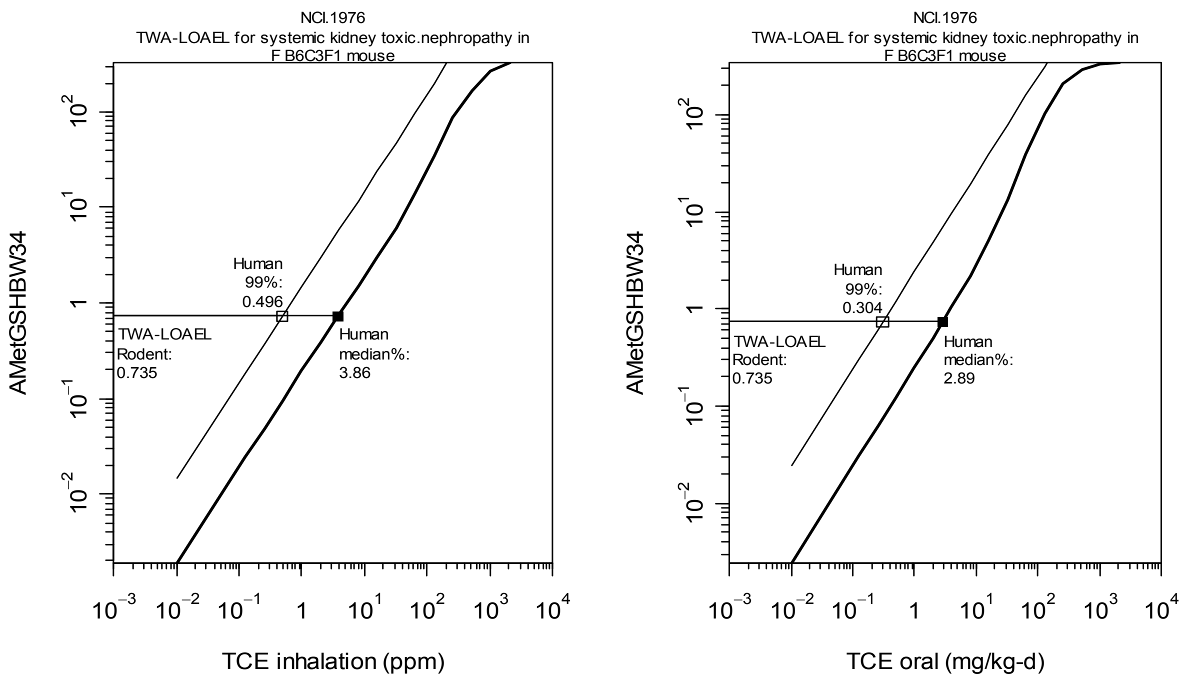


Figure F-12. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from NTP (1988) toxic nephrosis in mice.

F.6.3. Woolhiser et al. (2006)—Benchmark Dose (BMD) Modeling of Increased Kidney Weight in Rats

The critical endpoint here is increased kidney weights in female Sprague-Dawley (S-D) rats (Woolhiser et al., 2006).

F.6.3.1. *Dosimetry and Benchmark Dose (BMD) Modeling*

Rats were exposed to 100, 300, and 1000, 6 hours/day, 5 days/week, for 4 weeks. The primary dose metric was selected to be average amount of DCVC bioactivated/kg$^{3/4}$/day, with median estimates from the PBPK model for this study of 0.038, 0.10, and 0.51.

Figure F-13 shows BMD modeling for the continuous models used (see Section F.5.2, above). The Hill model with constant variance was selected because it had the lowest AIC and because other models with the same AIC either were a power model with power parameter <1 or had poor fits to the control data set.

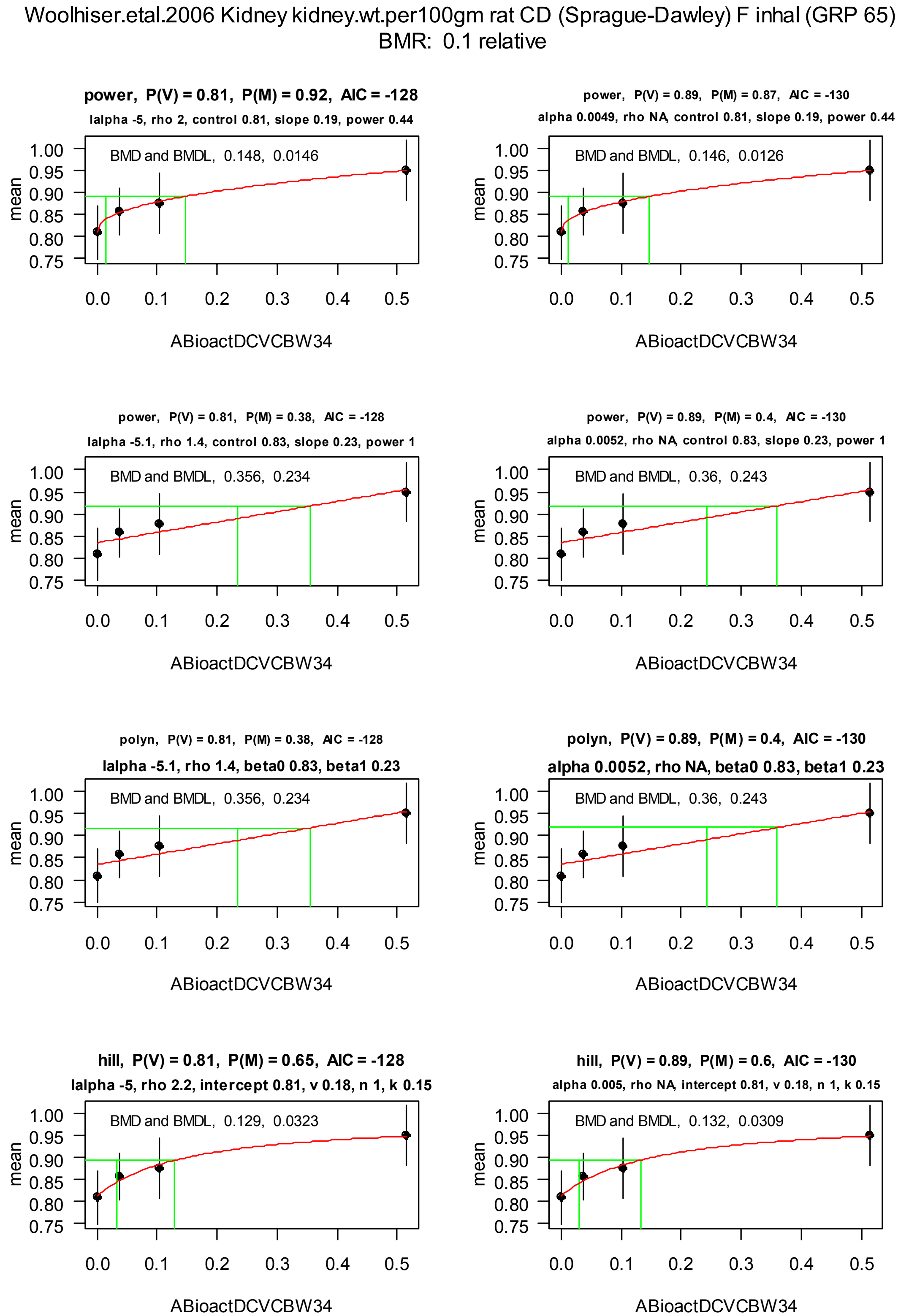


Figure F-13. BMD modeling of Woolhiser et al. (2006) for increased kidney weight in female S-D rats.

The idPOD of 0.0309 mg DCVC bioactivated/kg$^{3/4}$/day was a BMDL for a BMR of 10% weight change, which is the BMR typically used by U.S. EPA for body weight and organ weight changes. The response used in each case was the organ weight as a percentage of body weight, to account for any commensurate decreases in body weight, although the results did not differ much when absolute weights were used instead.

F.6.3.2. *Derivation of HEC$_{99}$ and HED$_{99}$*

The HEC$_{99}$ and HED$_{99}$ are the lower 99th percentiles for the continuous human exposure concentration and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC$_{99}$ of 0.0131 ppm and HED$_{99}$ of 0.00791 mg/kg/d for the 99th percentile for uncertainty and variability are shown in Figure F-14. These values are used as this critical effect's POD to which additional UFs are applied.

F.6.4. Keil et al. (2009)—Lowest-Observed-Adverse-Effect Level (LOAEL) for Decreased Thymus Weight and Increased Anti-dsDNA and Anti-ssDNA Antibodies in Mice

The critical endpoints here are decreased thymus weight and increased anti-dsDNA and anti-ssDNA antibodies in female B6C3F1 mice (Keil et al., 2009).

F.6.5. Keil et al. (2009)—Lowest-Observed-Adverse-Effect Level (LOAEL) for Decreased Thymus Weight and Increased Anti-dsDNA and Anti-ssDNA Antibodies in Mice

The critical endpoints here are decreased thymus weight and increased anti-dsDNA and anti-ssDNA antibodies in female B6C3F1 mice (Keil et al., 2009).

F.6.5.1. *Dosimetry*

Mice were exposed to 1400 and 14000 ppb of TCE in drinking water, with an average dose estimated by the authors to be 0.35 and 3.5 mg/kg/d, for 30 weeks. The dose-response relationships were sufficiently supralinear that BMD modeling failed to produce an adequate fit. The primary dose metric was selected to be the average amount of TCE metabolized/kg$^{3/4}$/day. The lower dose group was the LOAEL for both effects, and the median estimate from the PBPK model at that exposure level was 0.139 mg TCE metabolized/kg$^{3/4}$/day, which is used as the rodent idPOD.

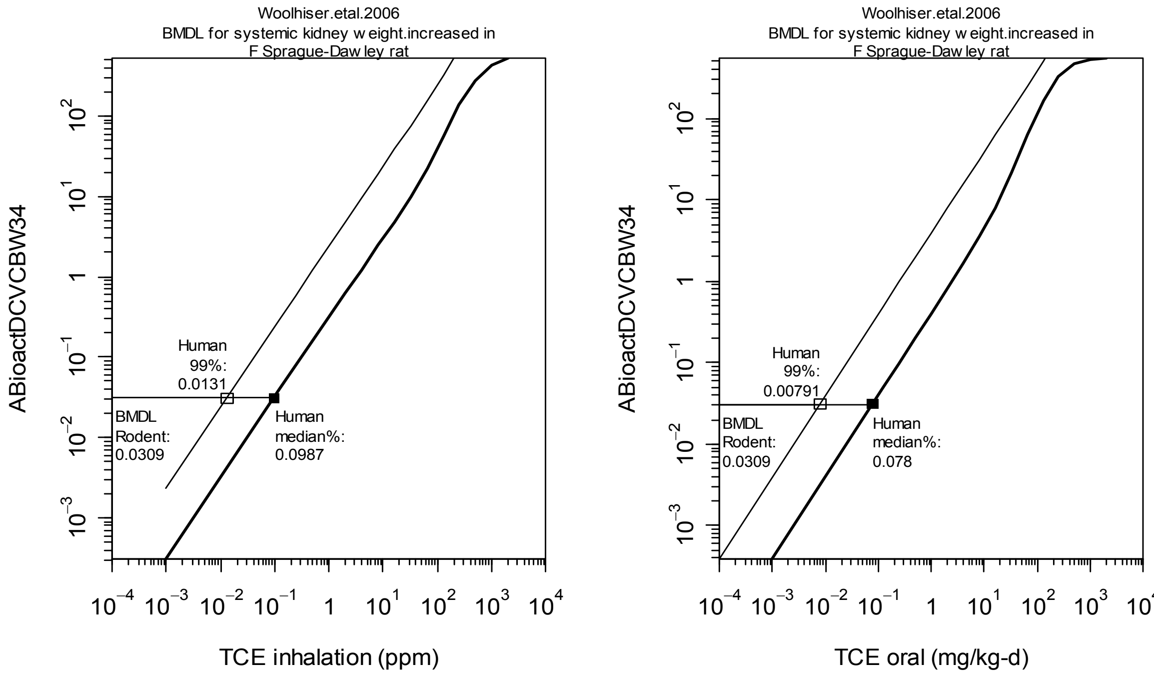


Figure F-14. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from Woolhiser et al. (2006) for increased kidney weight in rats.

F.6.5.2. *Derivation of HEC_{99} and HED_{99}*

The HEC_{99} and HED_{99} are the lower 99th percentiles for the continuous human exposure concentration and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.0332 ppm and HED_{99} of 0.0482 mg/kg/d for the 99th percentile for uncertainty and variability are shown in Figure F-15. These values are used as this critical effect's POD to which additional UFs are applied.

F.6.6. Johnson et al. (2003)—Benchmark Dose (BMD) Modeling of Fetal Heart Malformations in Rats

The critical endpoint here is increased fetal heart malformations in female S-D rats (Johnson et al., 2003).

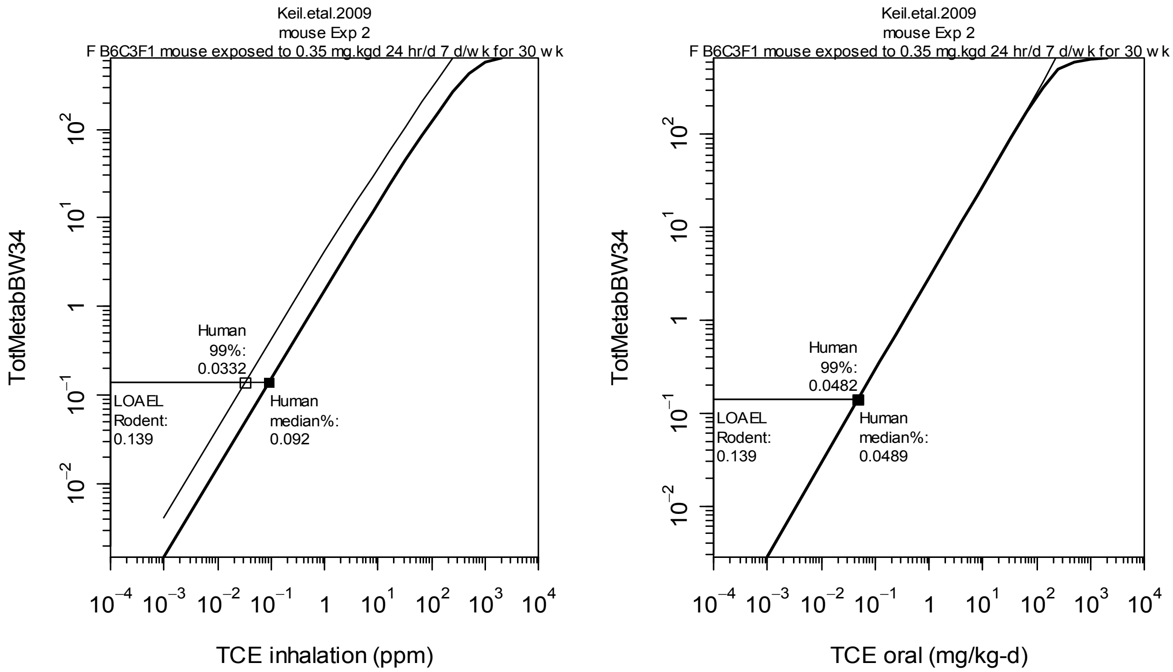


Figure F-15. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from Keil et al. (2009) for decreased thymus weight and increased anti-dsDNA and anti-ssDNA antibodies in mice.

F.6.6.1. *Dosimetry and Benchmark Dose (BMD) Modeling*

Rats were exposed to 2.5, 250, 1.5, or 1,100 ppm TCE in drinking water for 22 days (GD 1−22). The primary dose metric was selected to be average amount of TCE metabolized by oxidation/kg$^{3/4}$/day, with median estimates from the PBPK model for this study of 0.00031, 0.033, 0.15, and 88.

As discussed previously in Section F.4.2.1, from results of nested log-logistic modeling of these data, with the highest dose group dropped, the idPOD of 0.0142 mg TCE metabolized by oxidation/kg$^{3/4}$/day was a BMDL for a BMR of 1% increased in incidence in pups. A 1% extra risk of a pup having a heart malformation was used as the BMR because of the severity of the effect; some of the types of malformations observed could have been fatal.

F.6.6.2. *Derivation of HEC_{99} and HED_{99}*

The HEC_{99} and HED_{99} are the lower 99th percentiles for the continuous human exposure concentration and continuous human ingestion dose that lead to a human internal dose equal to the rodent idPOD. The derivation of the HEC_{99} of 0.00365 ppm and HED_{99} of 0.00515 mg/kg/d for the 99th percentile for uncertainty and variability are shown in Figure F-16. These values are used as this critical effect's POD to which additional UFs are applied.

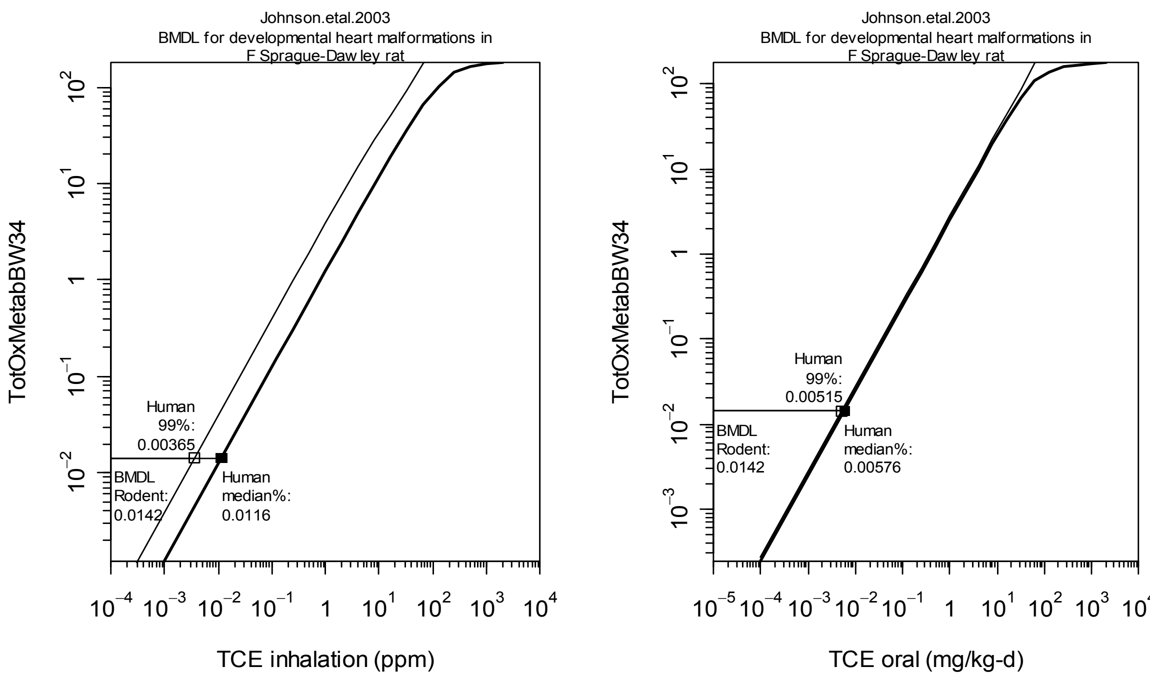


Figure F-16. Derivation of HEC_{99} and HED_{99} corresponding to the rodent idPOD from Johnson et al. (2003) for increased fetal cardiac malformations in female S-D rats using the total oxidative metabolism dose metric.

F.6.7. Peden-Adams et al. (2006)—Lowest-Observed-Adverse-Effect Level (LOAEL) for Decreased PFC Response and Increased Delayed-Type Hypersensitivity in Mice

The critical endpoints here are decreased PFC response and increased delayed-type hypersensitivity in mice exposed pre- and postnatally (Peden-Adams et al., 2006).

Mice were exposed to 1400 and 14,000 ppb in drinking water, with an average dose in the dams estimated by the authors to be 0.37 and 3.7 mg/kg/d, from GD0 to postnatal ages of 3 or 8 weeks. The dose-response relationships were sufficiently supralinear that BMD modeling failed to produce an adequate fit. In addition, because of the lack of an appropriate PBPK model and parameters to estimate internal doses given the complex exposure pattern (placental and lactational transfer, and pup ingestion postweaning), no internal dose estimates were made. Therefore, the LOAEL of 0.37 mg/kg/d on the basis of applied dose was used as the critical effect's POD to which additional UFs are applied.

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APPENDIX G

TCE Cancer Dose-Response Analyses with Rodent Cancer Bioassay Data

CONTENTS—Appendix G: TCE Cancer Dose-Response Analyses With Rodent Cancer Bioassay Data

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APPENDIX G: TCE CANCER DOSE-RESPONSE ANALYSES WITH RODENT CANCER BIOASSAY DATA

G.1. DATA SOURCES

Trichloroethylene (TCE) cancer endpoints were identified in Maltoni et al. (1986), National Cancer Institute (NCI, 1976), National Toxicology Program (NTP, 1988, 1990), Fukuda et al. (1983), and Henschler et al. (1980). These data were reviewed and tabulated in spreadsheets, and the numbers were verified. All endpoint data identified by authors as having a statistically significant response to dose were tabulated, and data that had marginally significant trends with dose were also reviewed. For all endpoints for which dose-response model estimates were presented, trends were verified using the Cochran-Armitage or the Poly-3 test.

G.1.1. Numbers at Risk

The numbers of animals at risk are not necessarily those used by the authors; instead, as the number at risk, the number alive at 52 weeks was used (if the first cancer of the type of interest was observed at later than 52 weeks) or the number alive at the week when the first cancer of the type of interest was observed. In general, the data of Maltoni et al. (1986) were presented in this way, in their tables titled "Incidence of the different types of tumors referred to specific corrected numbers." In a few cases in Maltoni et al. (1986), the time of first occurrence was later than 52 weeks, so an alternative number at risk was used from another column (for another cancer) in the same table having a first occurrence close to 52 weeks. For NTP (1988, 1990) and for NCI (1976), the week of the first observation and the numbers alive at that week were determined from the appendix tables. For Fukuda et al. (1983), the reported "effective number of mice" in their Table 2 was used, which is consistent with numbers alive at 40−42 weeks (when the first tumor, a thymic lymphoma, was observed) in their mortality curve. For Henschler et al. (1980), the number of mice alive at Week 36 (from their Figure 1), which is when the first tumor was observed (according to their Figure 2), was used.

G.1.2. Cumulative Incidence

Maltoni et al. (1986) conducted a lifetime study, in which rodents were exposed for 104 weeks (rats) or 78 weeks (mice), and allowed to live until they died "naturally." Maltoni et al. (1986) reported cumulative incidence on this basis, and it was not possible for us to determine incidence at any fixed time such as 104 weeks on study. For Henschler et al. (1980), the number of mice with tumors observed by Week 104 (their Figure 2) was used. The cumulative incidence reported by Fukuda et al. (1983) at 107 weeks (after 104 weeks of exposure) was used. For the NCI (1976) and NTP (1988, 1990) studies, the reported cumulative incidence at 103 to 107 weeks (study time varied by study and species) was used.

G.2. INTERNAL DOSE METRICS AND DOSE ADJUSTMENTS

Physiologically based pharmacokinetic (PBPK) modeling was used to estimate levels of dose metrics corresponding to different exposure scenarios in rodents and humans (see Section 3.5). The selection of dose metrics for specific organs and endpoints is discussed under Section 5.2. Internal dose metrics were selected based on applicability to each major affected organ. The dose metrics used with our cancer dose-response analyses are shown in Table G-1.

Table G-1. Internal dose metrics used in dose-response analyses, identified by "X"

Dose metric units	Liver	Lung	Kidney	Other
ABioactDCVCBW34 (mg/wk-kg$^{3/4}$)	0	0	X	0
AMetGSHBW34 (mg/wk-kg$^{3/4}$)	0	0	X	0
AMetLiv1BW34 (mg/wk-kg$^{3/4}$)	X	0	0	0
AMetLngBW34 (mg/wk-kg$^{3/4}$)	0	X	0	0
AUCCBld (mg-hr/L-wk)	0	X	0	X
TotMetabBW34 (mg/wk-kg$^{3/4}$)	0	0	X	X
TotOxMetabBW34 (mg/wk-kg$^{3/4}$)	X	X	0	0

The PBPK model requires the rodent body weight as an input. For most of the studies, central estimates specific to each species, strain, and sex (and substudy) were used. These were estimated by medians of body weights digitized from graphics in Maltoni et al. (1986), by medians of weekly averages in NTP (1990, 1988), and by averages over the study duration of weekly mean body weights tabulated in NCI (1976).

For the studies by Fukuda et al. (1983) and Henschler et al. (1980), mouse body weights were not available. After reviewing body weights reported for similar strains by two laboratories[1] and in the other studies reported for TCE, it was concluded that a plausible range for lifetime average body weight is 20–35 g, with a median near 28 g. For these two studies,

[1]http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=meas%2Fdatalister&req=Cbody+weight&pan=2&noomit=&datamode=measavg, http://www.hilltoplabs.com/public/growth.html.

internal dose metrics for these three average body weights (20, 28, and 35 g) were computed. The percentage differences between the internal dose metrics for the intermediate body weight (BW) of 28 g and the low and high average BW of 20 gm and 35 g were then evaluated. Internal dose metrics were little affected by choice of body weight. For all dose metrics, the differences were less than ±13%. A body weight of 28 g was used for these two studies.

The medians (from the Markov chain Monte Carlo posterior distribution) for each of the dose metrics for the rodent were used in quantal dose-response analyses. The median is probably the most appropriate posterior parameter to use as a dose metric, as it identifies a "central" measure and it is also a quantile, making it more useful in nonlinear modeling. The "multistage" dose-response functions are nonlinear. One is interested in estimating the expected response. The expected value of a nonlinear function of dose is under- or overestimated when the mean (expected value) of the dose is used, depending on whether the function is concave or convex. (This is Jensen's Inequality: for a real convex function f(X), $f[E(X)] \leq E[f(X)]$.) For the dose-response function, one is interested in E[f(X)], so using E(X) (estimated by the posterior mean) as the dose metric will not necessarily predict the mean response. Using the posterior median rather than the mean as the dose metric should lead to a response function that is closer to the median response. However, if the estimated dose-response function is close to linear, this source of distortion may be small, and the mean response might be predicted reasonably well by using the posterior mean as the dose metric. The mean and median are expected to be rather different because the posterior distributions are skewed and approximately lognormal. Therefore, results based on the posterior median and the posterior mean dose metric were compared before deciding to use the median.

G.3. DOSE ADJUSTMENTS FOR INTERMITTENT EXPOSURE

The nominal applied dose was adjusted for exposure discontinuity (e.g., exposure for 5 days per week and 6 hours per day reduced the dose by the factor [(5/7) * (6/24)]), and for exposure durations less than full study time (up to 2 years) (e.g., the dose might be reduced by a factor [78 wk/104 wk]). The PBPK dose metrics took into account the daily and weekly discontinuity to produce an equivalent dose for continuous exposure. The NCI (1976) gavage study applied one dose for weeks 1–12 and another, slightly different dose for weeks 13–78; PBPK dose metrics were produced for both dose regimes and then time-averaged (e.g., average dose = $(12/78) \times D1 + (66/78) \times D2$). For Henschler et al. (1980), Maltoni et al. (1986), and NCI (1976), a further adjustment of (exposure duration/study duration) was made to account for the fact that exposures ended prior to terminal sacrifice, so that the dose metrics reflect average weekly values over the exposure period. Finally, for NCI (1976), the dose metrics were then adjusted for early sacrifice[2] (at 91 weeks rather than 104 weeks) by a factor of $(91\ \text{wk}/104\ \text{wk})^3$.[3]

G.4. RODENT TO HUMAN DOSE EXTRAPOLATION

Adjustments for rodent-to-human extrapolation were applied to the final results—the benchmark dose (BMD), benchmark dose lower bound (BMDL), and cancer slope factor (potency), which is calculated as benchmark response (BMR)/BMDL, e.g., $0.10/\text{BMDL}_{10}$.

For the PBPK dose metrics, a ratio between human and laboratory animal internal dose was determined by methods described in Section 3.5. The cancer slope factor is relevant only for very low extra risk (typically on the order of 10^{-4} to 10^{-6}), thus very low dose, and it was determined that the relation between human and animal internal dose was linear in the low-dose range for each of the dose metrics used, hence this ratio was multiplied by the animal dose (or divided into the cancer slope factor) to extrapolate animal to human dose or concentration.

For the experimentally applied dose, default interspecies extrapolation approaches were used. These are provided for comparison to results based on PBPK metrics. To extrapolate animal inhalation exposure to human inhalation exposure, the “equivalent” human exposure concentration (i.e., the exposure concentration in humans that is expected to give the same level of response that was observed in the test species) was assumed to be identical to the animal inhalation exposure concentration, i.e., “ppm equivalence.” This assumption is consistent with U.S. Environmental Protection Agency recommendations (U.S. EPA, 1994) for deriving a human equivalent concentration for a Category 3 gas for which the blood:air partition coefficient in laboratory animals is greater than that in humans (see Section 3.1 for discussion of the TCE blood:air partition coefficient). To extrapolate animal oral exposure to equivalent human oral exposure, animal dose was scaled up by body weight to the ¾-power using the factor $(BW_{Human}/BW_{Animal})^{0.75}$. To extrapolate animal inhalation exposure to human oral exposure, the following equation (Eq. G-1) was used;[4]

[2]For studies of less than 2 years (i.e., with terminal kills before 2 years), the doses are generally adjusted by the study length ratio to a power of three (i.e., a factor $[\text{length of study in wk}/104\ \text{wk}]^3$) to reflect the fact that the animals were not observed for the full standard lifetime (U.S. EPA, 1980).

[3]For studies of less than 2 years (i.e., with terminal kills before 2 years), the doses are generally adjusted by the study length ratio to a power of three (i.e., a factor $[\text{length of study in wk}/104\ \text{wk}]^3$) to reflect the fact that the animals were not observed for the full standard lifetime (U.S. EPA, 1980).

[4]ToxRisk version 5.3, © 2000–2001 by the KS Crump Group, Inc.

Animal, equivalent oral intake, mg/kg/d =

$$ppm * [MW_{TCE}/24.45]^5 * MV * (60\ min/hr) * (10^3\ mg/g) * [24\ hr/BW_{kg}] \quad \text{(Eq. G-1)}$$

with units

$$ppm * [g/mol \div L/mol\ ] * L/min * (min/hr) * (mg/g) * [hr/day \div kg] \quad \text{(Eq. G-2)}$$

which reduces to

$$ppm * [7.738307 * MV/BW_{kg}] \quad \text{(Eq. G-3)}$$

where

ppm = animal inhalation concentration, $1/10^6$, unitless

MV = minute volume (breathing rate) at rest, L/minute.

Minute volume (MV) was estimated using equations from U.S. EPA (1994, p. 4–27),

$$\text{Mouse} \quad \ln(MV) = 0.326 + 1.05 * \ln(BW_{kg}) \quad \text{(Eq. G-4)}$$

$$\text{Rat} \quad \ln(MV) = -0.578 + 0.821 * \ln(BW_{kg}). \quad \text{(Eq. G-5)}$$

Animal equivalent oral intake was converted to human equivalent oral intake by multiplying by the rodent to human ratio of body weights to the power +0.25.[6]

To extrapolate animal oral exposure to equivalent human inhalation exposure, the calculation above was reversed to extrapolate the animal inhalation exposure.

G.5. COMBINING DATA FROM RELATED EXPERIMENTS IN MALTONI ET AL. (1986)

Data from Maltoni et al. (1986) required decisions by us regarding whether to combine related experiments for certain species and cancers.

In experiment BT306, which used B6C3F1 mice, males experienced unusually low survival, reportedly because of the age of the mice at the outset and resulting aggression. The

[5]Molecular weight of TCE is 131.39; there are 24.45 L of perfect gas per g-mol at standard temperature and pressure, U.S. EPA (1994).

[6]Find whole animal intake from mg/kg/d * BW_{Animal}. Scale this allometrically by $(BW_{Human}/BW_{Animal})^{0.75}$ to extrapolate whole human intake. Divide by human body weight to find mg/kg/d for the human. The net effect is Animal mg/kg/d * (BW_{Animal}/BW_{Human})^0.25 = Human mg/kg/d.

protocol was repeated (for males only), with an earlier starting age, as experiment BT306bis, and male survival was higher (and typical for such studies). The rapid male mortality in experiment BT306 apparently censored later-developing cancers, as suggested by the low frequency of liver cancers for males in BT306 as compared to BT306bis. Data for the two experiments clearly cannot legitimately be combined. Therefore only experiment BT306bis males were used in the analyses.

Experiments BT304 and BT304bis, on rats, provide evidence in male rats of leukemia, carcinomas of the kidney, and testicular (Leydig cell) tumors, and provide evidence in female rats for leukemia. Maltoni et al. (1986, p. 46) stated "Since experiments BT 304 and BT 304bis on Sprague-Dawley rats were performed at the same time, exactly in the same way, on animals of the same breed, divided by litter distribution within the two experiments, they have been evaluated separately and comprehensively." The data were also analyzed separately and in combination.

The data and modeling results for these tumors in the BT304 and BT304bis experiments are tabulated in Tables G-2 through G-5, below. It was decided that it was best to combine the data for the two experiments. There were no consistent differences between experiments, and no firm basis for selecting one of them. Our final analyses are, therefore, based on the combined numbers and tumor responses for these two experiments.

G.6. DOSE-RESPONSE MODELING RESULTS

Using BenchMark Dose Software (BMDS), the multistage quantal model was fitted using the applicable dose metrics for each combination of study, species, strain, sex, organ, and BMR (extra risk) value under consideration. A multistage model of order one less than the number of dose groups (g) was fitted. This means that in some cases the fitted model could be strictly nonlinear at low dose (estimated coefficient "b1" was zero), and in other cases, higher-order coefficients might be estimated as zero so the resulting model would not necessarily have order (#groups-1). Because more parsimonious, 1st-order models often fit such data well, based on our extensive experience and that of others (Nitcheva et al., 2007), if the resulting model was not a 1st-order multistage, then lower-order models were also fitted, down to a 1st-order multistage model. This permitted us to screen results efficiently.

Table G-2. Experiments BT304 and BT304bis, female Sprague-Dawley rats, Maltoni et al. (1986). Number alive is reported for week of first tumor observation in either males or females.[a] These data were not used for dose-response modeling because there is no consistent trend (for the combined data, there is no significant trend by the Cochran-Armitage test, and no significant differences between control and dose groups by Fisher's exact test).

Exposure Concen. (ppm)	No. alive	No. rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304, female rats, leukemias, *N* alive at 7 weeks							
0	105	7	0.067	No adequately fitting model				
100	90	6	0.067					
300	90	0	0.000					
600	90	7	0.078					
	Experiment BT304bis, female rats, leukemias, *N* alive at 7 weeks							
0	40	0	0.000	1	0.202	70.4	127	58.7
100	40	3	0.075					
300	40	2	0.050					
600	40	4	0.100					
	Experiments BT304 and BT304bis, female rats, leukemias, combined data							
0	145	7	0.048	3	0.081	227	180	134
100	130	9	0.069					
300	130	2	0.015					
600	130	11	0.085					

[a] First tumor occurrences were not reported separately by sex.
[b] Models of orders 3 were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24) * (5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations).

AIC – Akaike Information Criteria.

Table G-3. Experiments BT304 and BT304bis, male Sprague-Dawley rats, Maltoni et al. (1986): leukemias. Number alive is reported for week of first tumor observation in either males or females.[a]

Exposure concen. (ppm)	No. alive	No. rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304, male rats, leukemias, *N* alive at 7 weeks							
0	95	6	0.063	1	0.429	238	NA	NA
100	90	10	0.111					
300	90	11	0.122					
600	89	9	0.101					
	Experiment BT304bis, male rats, leukemias, *N* alive at 7 weeks							
0	39	3	0.077	3	0.979	102	143	71.9
100	40	3	0.075					
300	40	3	0.075					
600	40	6	0.150					
	Combined data for BT304 and BT304bis, male rats, leukemias							
0	134	9	0.067	1	0.715	337	269	111
100	130	13	0.100					
300	130	14	0.108					
600	129	15	0.116					

[a]First tumor occurrences were not reported separately by sex.
[b]Models of orders 3 were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24)*(5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations). "NA" indicates the BMD or BMDL could not be solved because it exceeded the highest dose.

AIC—Akaike Information Criteria.

Table G-4. Experiments BT304 and BT304bis, male Sprague-Dawley rats, Maltoni et al. (1986): kidney adenomas + carcinomas. Number alive is reported for week of first tumor observation in either males or females.[a]

Exposure concen. (ppm)	No. alive	No. rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304 male rats, kidney adenomas + carcinomas, *N* alive at 47 weeks							
0	87	0	0.000	3	0.318	50.1	173	134
100	86	1	0.012					
300	80	0	0.000					
600	85	4	0.047					
	Experiment BT304bis, male rats, kidney adenomas + carcinomas, *N* alive at 53 weeks							
0	34	0	0.000	3	0.988	13.0	266	173
100	32	0	0.000					
300	36	0	0.000					
600	38	1	0.027					
	Combined data for BT304 and BT304bis, male rats, kidney adenomas + carcinomas							
0	121	0	0.000	3	0.292	60.5	181	144
100	118	1	0.008					
300	116	0	0.000					
600	123	5	0.041					

[a] First tumor occurrences were not reported separately by sex.
[b] Models of orders three were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24)*(5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations). "NA" indicates the BMD or BMDL could not be solved because it exceeded the highest dose.

AIC – Akaike Information Criteria.

Table G-5. Experiments BT304 and BT304bis, male Sprague-Dawley rats, Maltoni et al. (1986): testis, Leydig cell tumors. Number alive is reported for week of first tumor observation.[a]

Exposure concen. (ppm)	No. alive	No. rats with this cancer	Proportion with cancer	Multistage model fit statistics[b]				
				Model order	*p*-Value	AIC	BMD_{10}	$BMDL_{10}$
	Experiment BT304, male rats, Leydig cell tumors, *N* alive at 47 weeks							
0	87	5	0.057	1	0.0494	309	41.5	29.2
100	86	11	0.128					
300	80	24	0.300					
600	85	22	0.259					
	Experiment BT304bis, male rats, Leydig cell tumors, *N* alive at 53 weeks							
0	34	1	0.029	1	0.369	117	54.5	30.9
100	32	5	0.156					
300	36	6	0.167					
600	38	9	0.237					
	Combined data for BT304 and BT304bis, male rats, Leydig cell tumors							
0	121	6	0.050	1	0.0566	421	44.7	32.7
100	116	16	0.138					
300	116	30	0.259					
600	122	31	0.254					

[a] Numbers alive reported for weeks as close as possible to Week 52 (first tumors observed at weeks 81, 62, respectively, for the two experiments).
[b] Models of orders three were fitted; the highest-order nonzero coefficient is reported in column "Model order." BMDL was estimated for extra risk of 0.10 and confidence level 0.95. Exposure concentrations were multiplied by (7/24)*(5/7) = 0.20833 before fitting the models, to adjust for exposure periodicity (i.e., the time-averaged concentrations were about 20% of the nominal concentrations). "NA" indicates the BMD or BMDL could not be solved because it exceeded the highest dose.

AIC – Akaike Information Criteria.

The document Appendix.linked.files\AppG.Cancer.Rodents.Plots.TCE.DRAFT.pdf shows the fitted model curves. The graphics include observations (as proportions, i.e., cumulative incidence divided by number at risk), the estimated multistage curve (solid red line) and estimated BMD, with a BMDL. Vertical bars show 95% confidence intervals for the observed proportions. Printed above each plot are some key statistics (necessarily rounded) for model goodness of fit and estimated parameters. Printed in the plots at upper left are the BMD and BMDL for the rodent data, in the same units as the rodent dose. Within the plot at lower right are human exposure values (BMDL and cancer slope factor for continuous inhalation and oral exposures) corresponding to the rodent BMDL. For applied doses, the human equivalent values were calculated by "default" methods,[7] as discussed above, and then only for the same route of exposure as the rodent, and they are in units of rodent dose. For internal dose metrics, the human values are based upon the PBPK rodent-to-human extrapolation, as discussed in Section 5.2.1.2.

The document Appendix.linked.files\AppG.Cancer.Rodents.Results.TCE.DRAFT.pdf presents the data and model summary statistics, including goodness-of-fit measures (Chi-square goodness-of-fit *p*-value, Akaike Information Criteria [AIC]), parameter estimates, BMD, BMDL, and "cancer slope factor" ("CSF"), which is the extra risk divided by the BMDL. Much more descriptive information appears also, including the adjustment terms for intermittent exposure, and the doses before applying those adjustments. The group "GRP" numbers are arbitrary, and are the same as GRP numbers in the plots. There is one line in this table for each dose-response graph in the preceding document. Input data for the analyses are in the file Appendix.linked.files\AppG.Cancer.Rodents.Input.Data.TCE.DRAFT.pdf. Finally, the values and model selections for the results used in Section 5.2 are summarized in the file Appendix.linked.files\AppG.Cancer.Rodents.model.selections.TCE.DRAFT.pdf (primary dose metrics in bold).

G.7. MODELING TO ACCOUNT FOR DOSE GROUPS DIFFERING IN SURVIVAL TIMES

Differential mortality among dose groups can potentially interfere with (i.e., censor) the occurrence of late-appearing cancers. Usually the situation is one of greater mortality rates at higher doses, caused by toxic effects, or, sometimes, by cancers other than the cancer of interest. Statistical methods of estimation (for the cancer of interest) in the presence of competing risks assume uninformative censoring.

[7]For oral intake, dose (BMDL) is multiplied by the ratio of animal to human body weight (60 kg female, 70 kg male) taken to the ¼ power. For inhalation exposures, ppm equivalence is assumed.

For bioassays with differential early mortality occurring primarily before the time of the 1st tumor or 52 weeks (whichever came first), the effects of early mortality were largely accounted for by adjusting the tumor incidence for animals at risk, as described above, and the dose-response data were modeled using the multistage model.

If, however, there was substantial overlap between the appearances of cancers and progressively differential mortality among dose groups, it was necessary to apply methods that take into account individual animal survival times. Two such methods were used here: time-to-tumor modeling and the poly-3 method of adjusting numbers at risk. Three such studies were identified, all with male rats (see Table 5-27). Using both survival-adjustment approaches, BMDs and BMDLs were obtained and unit risks derived. Section 5.2.1.3 presents a comparison of the results for the three data sets and for various dose metrics.

G.7.1. Time-to-Tumor Modeling

The first approach used to take into account individual survival times was application of the multistage Weibull (MSW) time-to-tumor model. This model has the general form

$$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) * (t - t_0)^z], \qquad \text{(Eq. G-6)}$$

where $P(d,t)$ represents the probability of a tumor by age t for dose d, and parameters $z \geq 1$, $t_0 \geq 0$, and $q_i \geq 0$ for $i = 0,1,...,k$, where k = the number of dose groups; the parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death. The MSW model likelihood accounts for the left-censoring inherent in "Incidental" observations of nonfatal tumors discovered upon necropsy and the right-censoring inherent in deaths not caused by fatal tumors. All of our analyses used the model for incidental tumors, which has no t_0 term, and which assumes that the tumors are nonfatal (or effectively so, to a reasonable approximation). This seems reasonable because the tumors of concern appeared relatively late in life and there were multiple competing probable causes of death (especially toxic effects) operating in these studies (also note that cause of death was not reported by the studies used). It is difficult to formally evaluate model fit with this model because there is no applicable goodness-of-fit statistic with a well-defined asymptotic distribution. However, plots of fitted vs. observed responses were examined.

A computer program ("MSW") to implement the multistage Weibull time-to-tumor model was designed, developed and tested for U.S. EPA by Battelle Columbus (Ohio). The MSW program obtains maximum likelihood estimates for model parameters and solves for the BMDL (lower confidence limit for BMD) using the profile-likelihood method. The model, with documentation for methodology (statistical theory and estimation, and numerical algorithms) and testing, was externally reviewed by experts in June 2007. Reviews were generally positive and confirmed that the functioning of the computer code has been rigorously tested. (U.S. EPA and Battelle confirmed that MSW gave results essentially identical to those of "ToxRisk," a program no longer commercially issued or supported.) U.S. EPA's BMDS Web site provided reviewers' comments and U.S. EPA's responses.[8] The MSW program and reports on statistical and computational methodology and model testing will be made available in 2009 (after implementing some changes to reporting features and error-handling).

Results of this modeling are shown in the file Appendix.linked.files\AppG.Cancer.Rodents.TimetoTumor.Results.TCE.DRAFT.pdf.

G.7.2. Poly-3 Calculation of Adjusted Number at Risk

To obtain an independent estimate of a point of departure using different assumptions, it was thought desirable to compare time-to-tumor modeling to an alternative survival-adjustment technique, "poly-3 adjustment" (Portier and Bailer, 1989), applied to the same data. This technique was used to adjust the tumor incidence denominators based on the individual animal survival times. The adjusted incidence data then served as inputs for U.S. EPA's BMDS multistage model, and multistage model selection was conducted as described in Section 5.2.

A detailed exposition is given by Piegorsch and Bailer (1997), Section 6.3.2. Each tumor-less animal is weighted by its fractional survival time (survival time divided by the duration of the bioassay) raised to the power of 3 to reflect the fact that animals are at greater risk of cancer at older ages. Animals with tumors are given a weight of 1. The sum of the weights of all the animals in an exposure group yields the effective survival-adjusted denominator. The "default" power of 3 (thus, "poly-3") was assumed, which was found to be representative for a large number of cancer types (Portier et al., 1986). Algebraically,

$$N_{adj} = \sum_i w_i \qquad \text{(Eq. G-7)}$$

[8]At http://www.epa.gov/ncea/bmds/response.html under title "2007 External Review of New Quantal Models;" use links to comments and responses.

where

w_i = 1 if tumor is present
w_i = $(t_i/T)^3$ if tumor is absent at time of death (t_i)
T = duration of study. N was rounded to the nearest integer.[9]

Calculations are reproduced in the spreadsheets linked above.

G.8. COMBINED RISK FROM MULTIPLE TUMOR SITES

For bioassays that exhibited more than one type of tumor response in the same sex and species (these studies have a row for "combined risk" in the "Endpoint" column of Table 5-27, Section 5.2), the cancer potency for the different tumor types combined was estimated. The combined tumor risk estimate describes the risk of developing tumors for *an*y (not all together) of the tumor types that exhibited a TCE-associated tumor response; this estimate then represents the total excess cancer risk. The model for the combined tumor risk is also multistage, with the sum of the stage-specific multistage coefficients from the individual tumor models serving as the stage-specific coefficients for the combined risk model (i.e., for each q_i, $q_{i[combined]} = q_{i1} + q_{i2} + ... + q_{ik}$, where the q_is are the coefficients for the powers of dose and k is the number of tumor types being combined) (Bogen, 1990; NRC, 1994). This model assumes that the occurrences of two or more tumor types are independent. The resulting model equation can be readily solved for a given BMR to obtain a maximum likelihood estimate (BMD) for the combined risk. However, the confidence bounds for the combined risk estimate are not calculated by available modeling software. Therefore, a Bayesian approach was used to estimate confidence bounds on the combined BMD. This approach was implemented using the freely available WinBUGS software (Spiegelhalter et al., 2003), which applies Markov chain Monte Carlo computations. Use of WinBUGS has been demonstrated for derivation of a distribution of BMDs for a single multistage model (Kopylev et al., 2007) and can be straightforwardly generalized to derive the distribution of BMDs for the combined tumor load.

G.8.1. Methods

G.8.1.1. *Single Tumor Sites*

Cancer dose-response models were fitted to data using BMDS. These were multistage models with coefficients constrained to be non-negative. The order of model fitted was (g – 1),

[9]Notice that the assumptions required for significance testing and estimating variances of parameters are changed by this procedure. The Williams-Bieler variance estimator is described by Piegorsch and Bailer (1997). Our multistage modeling did not take this into account, so the resulting BMDL may be somewhat lower than could be obtained by more laborious calculations.

where g is the number of dose groups. For internal dose metrics, the values shown in tables above were used.

The multistage model was modified for U.S. EPA NCEA by Battelle (under contract EPC04027) to provide model-based estimates of extra risk at a user-specified dose and profile-likelihood confidence intervals for that risk. Thus, confidence intervals for extra risk in addition to BMDs could be reported.

G.8.1.2. *Combined Risk From Multiple Tumor Sites*

The multistage model identified by BMDS[10] was used in a WinBUGS script to generate posterior distributions for model parameters, the BMD and extra risk at the same dose specified for the BMDS estimates. The burn-in was of length 10,000, then 100,000 updates were made and thinned to every 10th update for sample monitoring. From a WinBUGS run, the sample histories, posterior distribution plots, summary statistics, and codas were archived.

Codas were then imported to R and processed using R programs to compute BMD and the extra risk at a specific dose for each tumor type. BMD and extra risk for the combined risk function (assuming independence) were also computed following Bogen.[11] Results were summarized as percentiles, means, and modes (modes were based upon the smoothed posterior distributions). The extra risks across tumor types at a specific dose (10 or 100 was used) were also summed.

BMDLs for rodent internal doses, reported below, were converted to human external doses using the conversion factors in Tables G-6 and G-7 (based on PBPK model described in Section 3.5).

Table G-6. Rodent to human conversions for internal dose metric TotOxMetabBW34

Route	Sex	Human (mean)
Inhalation, ppm	F	9.843477
	M	9.702822
Oral, mg/kg/d	F	15.72291
	M	16.4192

[10]The highest-order model was used, e.g., if BMDS estimates were gamma = 0, beta.1 > 0, beta.2 = 0, beta.3 > 0, the model in WinBUGS allowed beta.2 to be estimated (rather than being fixed at zero).

[11]Bogen, K.T. 1990. Uncertainty in Environmental Health Risk Assessment. London: Taylor & Francis [Chapter IV]. NRC (National Research Council). 1994. Science and Judgement in Risk Assessment. Washington, DC: National Academy Press [Chapter 11, Appendix I-1, Appendix I-2].

Table G-7. Rodent to human conversions for internal dose metric TotMetabBW34

Route	Sex	Human (mean)
Inhalation, ppm	F	11.84204
	M	11.69996
Oral, mg/kg/d	F	18.76327
	M	19.6

The application of rodent to human conversion factors is as follows:

Given rodent internal dose *D* in some units of TotOxMetabBW34, divide by tabled value *Y* above to find human exposure in ppm or mg/kg/d.

Example: ppm (human) = *D*(rodent)/*Y*

ppm (human female mean) = 500 (internal units)/9.843477

= 50.80 ppm (Eq. G-8)

G.8.2. Results

The results follow in this order:

Applied doses

NCI, 1976, Female B6C3F1 mice, oral gavage, liver and lung tumors and lymphomas (see Tables G-8 through G-10 and Figures G-1 and G-2)

Maltoni, 1986, Female B6C3F1 mice, inhalation (expt. BT306), liver and lung tumors (see Tables G-11 through G-13 and Figures G-3 and G-4)

Maltoni, 1986, Male Sprague-Dawley rats, inhalation (expt. BT304), kidney tumors, testis Leydig Cell tumors, and lymphomas (see Tables G-14 through G-16 and Figures G-5 and G-6)

Internal Doses

NCI, 1976, Female B6C3F1 mice, oral gavage, liver and lung tumors and lymphomas (see Tables G-17 through G-19 and Figures G-7 and G-8)

Maltoni, 1986, Female B6C3F1 mice, inhalation (expt. BT306), liver and lung tumors (see Tables G-20 through G-22 and Figures G-9 and G-10)

Maltoni, 1986, Male Sprague-Dawley rats, inhalation (expt. BT304), kidney tumors, Testis Leydig Cell tumors, and lymphomas (see Tables G-23 through G-25 and Figures G-11 and G-12)

Table G-8. Female B6C3F1 mice—applied doses: data

Dose[a]	N[b]	Liver hepatocellular carcinomas	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
0	18	0	1	1
356.4	45	4	4	5
713.3	41	11	7	6

[a] Doses were adjusted by a factor 0.41015625, accounting for exposure 5/7 days/week, exposure duration 78/91 weeks, and duration of study (91/104)^3. Averaged applied gavage exposures were low-dose 869 mg/kg/d, high dose 1,739 mg/kg/d.
[b] Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Source: NCI (1976).

Table G-9. Female B6C3F1 mice—applied doses: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order, *selected	Coeff. estimates equal zero	AIC	Largest* scaled residual	Goodness of fit p-value
Liver	2	γ	78.68	0	1
	1*	γ	77.52	–0.711	0.6698
Lung	2	NA	78.20	0	1
	1*	NA	76.74	–0.551	0.4649
Lymphomas + sarcomas	2	$\beta2$	77.28	0.113	0.8812
	1*	NA	77.28	0.113	0.8812

* Largest in absolute value.

Source: NCI (1976).

Table G-10. Female B6C3F1 mice—applied doses: BMD and risk estimates (inferences for BMR of 0.05 extra risk at 95% confidence level)

	Liver hepatocellular carcinomas	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
Parameters used in model	q0, q1	q0, q1	q0, q1
p-Value for BMDS model	0.6698	0.6611	0.8812
BMD_{05} (from BMDS)	138.4	295.2	358.8
BMD_{05} (median, mode—WinBUGS)	155.5, 135.4	314.5, 212.7	352.3, 231.7
BMDL (BMDS)*	92.95	144.3	151.4
BMDL (5^{th} percentile, WinBUGS)	97.48	150.7	157.7
BMD_{05} for combined risk (median, mode, from WinBUGS)	84.99, 78.95		
BMDL for combined risk (5^{th} percentile, WinBUGS)	53.61		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.03640	0.01722	0.01419
Upper 95% CL	0.05749	0.03849	0.03699
Sum of risks at dose 100	0.06781		
WinBUGS Bayes risk estimates			
Risk at dose 100: mean, median	0.0327, 0.0324	0.0168, 0.0161	0.0152, 0.0143
Upper 95% CL	0.0513	0.0334	0.0319
Comb. risk at dose 100 mean, median	0.06337, 0.0629		
Comb. risk at dose 100, upper 95% CL	0.09124		

* All confidence intervals are at 5% (lower) or 95% (upper) level, one-sided.

Source: NCI (1976).

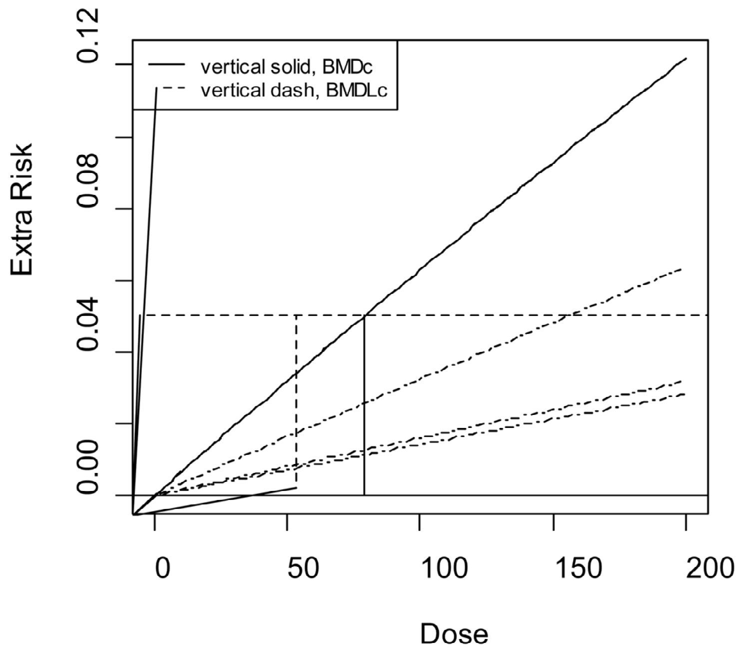

Figure G-1. Female B6C3F1 mice—applied doses: combined and individual tumor extra-risk functions.

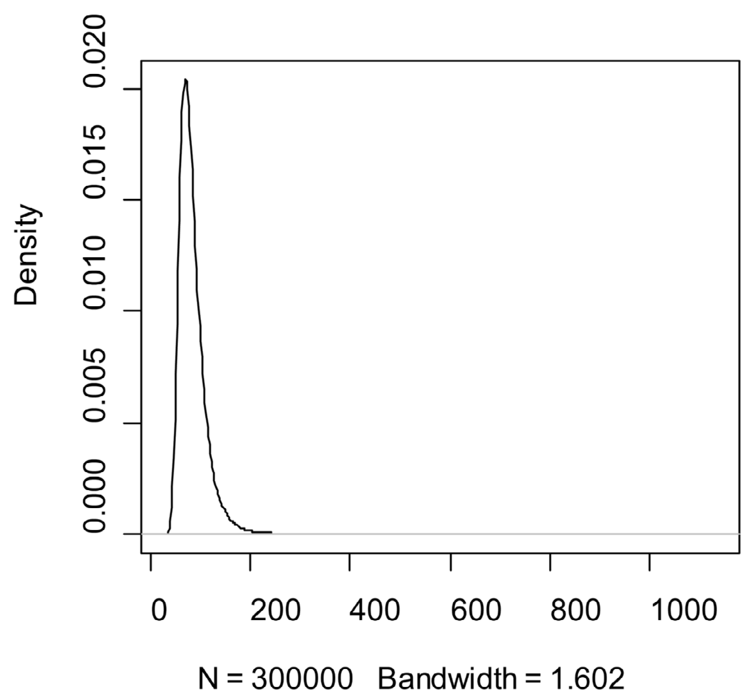

Figure G-2. Female B6C3F1 mice—applied doses: posterior distribution of BMDc for combined risk.

Table G-11. B6C3F1 female mice inhalation exposure—applied doses

Dose[a]		Liver hepatomas/*N* [b]	Lung adenomas + carcinomas/*N* [b]
0		3/88	2/90
15.6		4/89	6/90
46.9		4/88	7/89
93.8		9/85	14/87

[a] Doses adjusted by a factor 0.133928571, accounting for exposure 7/24 hours/day × 5/7 days/week, and exposure duration 78/104 weeks. Applied doses were 100, 300, and 600 ppm.
[b] Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Source: Maltoni (1986).

Table G-12. B6C3F1 female mice—applied doses: model selection comparison of model fit statistics for multistage models of increasing order

Tumor Site	Model order, *selected	Coeff. estimates equal zero	AIC	Largest* scaled residual	Goodness of fit *p*-value
Liver	3	β2	154.91	0.289	0.7129
	2	β1	153.02	0.330	0.8868
	1*	NA	153.47	–0.678	0.7223
Lung	3	β2	195.91	0.741	0.3509
	2	β2	193.91	0.714	0.6471
	1*	NA	193.91	0.714	0.6471

*Largest in absolute value.

Source: Maltoni (1986).

Table G-13. B6C3F1 female mice inhalation exposure—applied doses (inferences for 0.05 extra risk at 95% confidence level)

	Liver hepatomas	**Lung adenomas + carcinomas**
Parameters used in model	q0, q1	q0, q1
p-Value for BMDS model	0.7223	0.06471
BMD_{05} (from BMDS)	72.73	33.81
BMD_{05} (median, mode—WinBUGS)	71.55, 56.79	34.49, 31.65
BMDL (BMDS)*	37.13	21.73
ms_combo.exe BMD_{05}c, BMDLc	32.12, 16.22	
BMD_{05} (5^{th} percentile, WinBUGS)	37.03	22.07
BMD_{05} for combined risk (median, mode, from WinBUGS)	23.07, 20.39	
BMDL for combined risk (5^{th} percentile, WinBUGS)	15.67	
BMDS maximum likelihood risk estimates		
Risk at dose 10	0.0070281	0.0150572
Upper 95% CL	0.0151186	0.0250168
Sum of risks at dose 10	0.0220853	
WinBUGS Bayes risk estimates: means (medians)		
Risk at dose 10: mean, median	0.007377, 0.007138	0.01489, 0.01476
Upper 95% CL	0.01374	0.02
Comb. risk at dose 10: mean, median	0.02216, 0.02198	
Comb. risk at dose 10: upper 95% CL	0.03220	

* All confidence intervals are at 5% (lower) or 95% (upper) level, one-sided.

Source: Maltoni (1986).

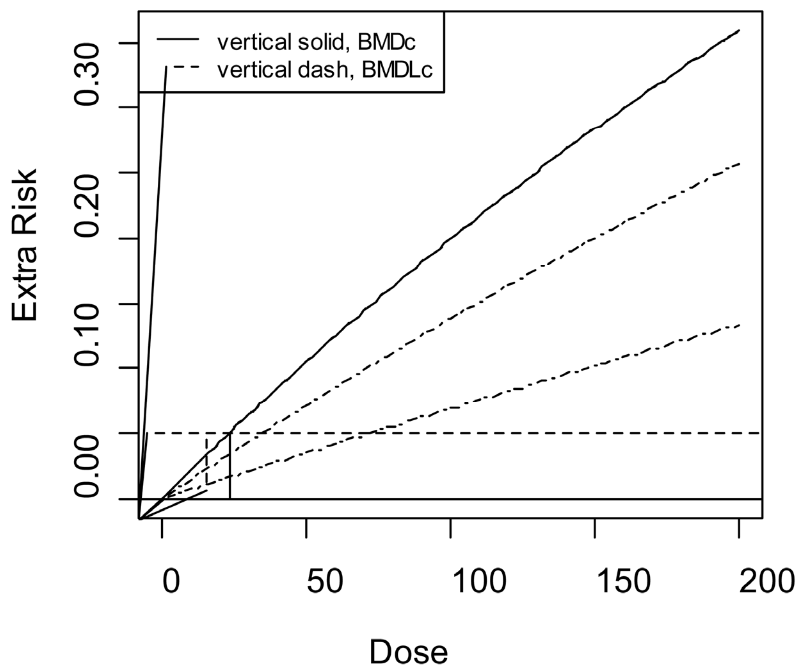

Figure G-3. B6C3F1 female mice inhalation exposure—applied doses: combined and individual tumor extra-risk functions.

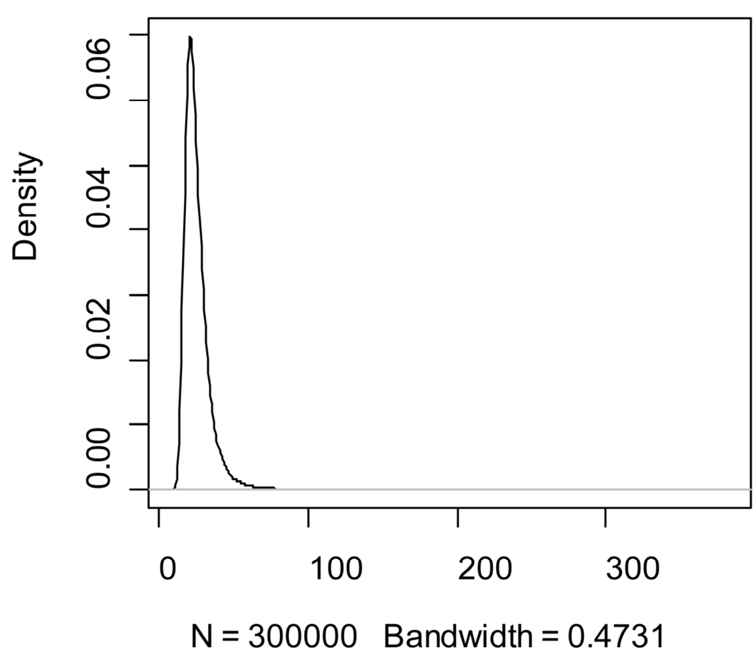

Figure G-4. B6C3F1 female mice inhalation exposure—applied doses: posterior distribution of BMDc for combined risk.

Table G-14. Maltoni Sprague-Dawley male rats—applied doses

Dose[a]		Kidney adenomas + carcinomas/N[b]	Leukemias/N[b]	Testis, Leydig cell tumors/N[b]
0		0/121	9/134	6/121
20.8		1/118	13/130	16/116
62.5		0/116	14/130	30/116
125		5/123	15/129	31/122

[a] Doses adjusted by a factor 0.208333333, accounting for exposure 7 hours/day × 5/7 days/week. Applied doses were 100, 300, and 600 ppm.
[b] Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Table G-15. Maltoni Sprague-Dawley male rats—applied doses: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order*	Coeff. estimates equal zero	AIC	Largest+ scaled residual	Goodness of fit *p*-value
Kidney	3	β1, β2	60.55	1.115	0.292
	2	γ	61.16	–1.207	0.253
	1*	γ	59.55	–1.331	0.4669
Leukemia	3	β2, β3	336.8	0.537	0.715
	2	β2	336.8	0.537	0.715
	1	NA	336.8	0.537	0.715
Dropping high dose	2	β2	243.7	0.512	0.529
	1*	NA	243.7	0.512	0.529
Testis	3	β2, β3	421.4	–1.293	0.057
	2	β2	421.4	–1.293	0.057
	1	NA	421.4	–1.293	0.057
Dropping high dose	2	β2	277.6	0.291	0.728
	1*	NA	277.6	0.291	0.728

* Model order selected + largest in absolute value

Table G-16. Maltoni Sprague-Dawley male rats—applied doses

	Kidney adenomas + carcinomas	Leukemia (high dose dropped)	Testis, Leydig cell tumors (high dose dropped)
Parameters used in models	q0, q1	q0, q1	q0, q1
p-Value for BMDS model	0.4669	0.5290	0.7277
BMD_{01} (from BMDS)	41.47	14.5854	2.46989
BMD_{01} (median, mode—WinBUGS)	46.00, 35.71	12.32, 8.021	2.497, 2.309
BMDL (BMDS)*	22.66	5.52597	1.77697
BMDL (5^{th} percentile, WinBUGS)	23.23	5.362	1.789
BMD_{01} for combined risk (median, mode, from WinBUGS)	1.960, 1.826		
BMDL for combined risk (5^{th} percentile, WinBUGS)	1.437		
BMDS maximum likelihood risk estimates			
Risk at dose 10	0.0024208	0.0068670	0.0398747
Upper 95% CL	0.0048995	0.0202747	0.0641010
Sum of risks at dose 10			
Risk at dose 1	0.0002423	0.0006888	0.0040609
Upper 95% CL	0.0004911	0.0020462	0.0066029
Sum of risks at dose 1			
WinBUGS Bayes risk estimates: means (medians)			
Risk at dose 10: mean, median	0.002302, 0.002182	0.008752, 0.008120	0.03961, 0.03945
Upper 95% CL	0.004316	0.01860	0.05462
Comb. risk at dose 10, mean, median	0.05020, 0.04998		
Comb. risk at dose 10, upper 95% CL	0.06757		
Risk at dose 1: mean, median	2.305e-04, 2.184e-04	8.800e-04, 8.150e04	0.004037, 0.004017
Upper 95% CL	4.325e-04	1.876e-03	0.005601
Comb. risk at dose 1, mean, median	0.005143, 0.005114		
Comb. risk at dose 1, upper 95% CL	0.006971		

* All confidence intervals are at 5% (lower) or 95% (upper) level, one-sided.

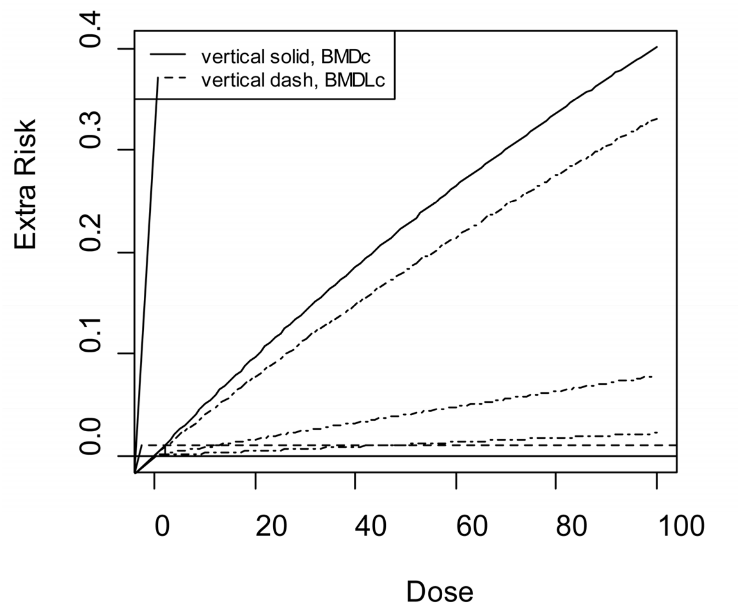

Figure G-5. Maltoni Sprague-Dawley male rats—applied doses: combined and individual tumor extra-risk functions.

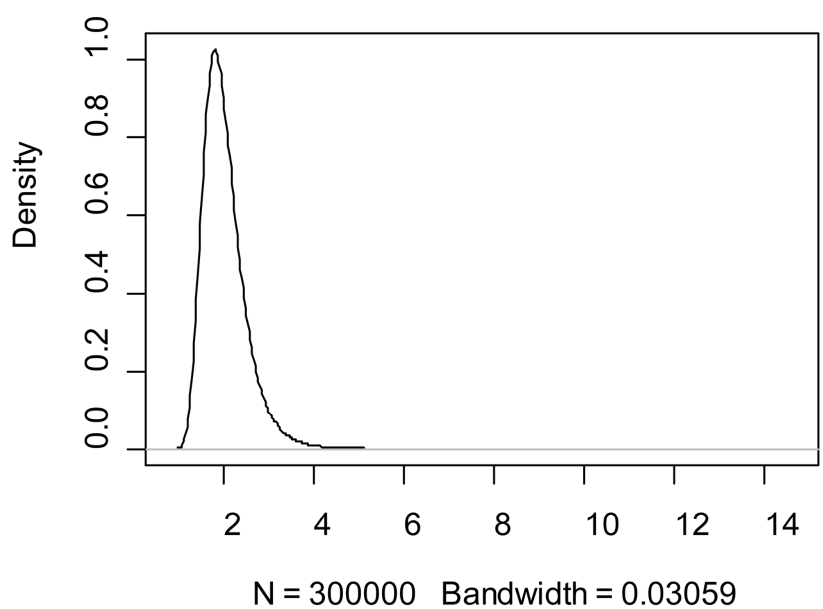

Figure G-6. Maltoni Sprague-Dawley male rats—applied doses: posterior distribution of BMDc for combined risk.

Table G-17. Female B6C3F1 mice—internal dose metric (total oxidative metabolism): data

Internal dose[a]	N[b]	Liver hepatocellular carcinomas	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
0	18	0	1	1
549.8	45	4	4	5
813.4	41	11	7	6

[a]Internal dose, Total Oxidative Metabolism, adjusted for body weight, units [mg/(wk-kg$^{3/4}$)]. Internal doses were adjusted by a factor 0.574219, accounting for exposure duration 78/91 weeks, and duration of study (91/104)^3. Before adjustment, the median internal doses were 957.48 and 1416.55 (mg/wk-kg$^{3/4}$).
[b]Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Source: NCI (1976).

Table G-18. Female B6C3F1 mice—internal dose: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	BMD, BMDL	Model order*	Coeff. estimates equal zero	AIC	Largest+ scaled residual	Goodness of fit *p*-value
Liver	505, 284	2*	γ, β1	77.25	–0.594	0.7618
	367, 245	1	γ	78.86	–1.083	0.3542
Lung	742, 396	2*	β1	76.33	–0.274	0.7197
	780, 380	1	NA	76.74	–0.551	0.4649
Lymphomas + sarcomas	870, 389	2	NA	79.26	0	1
	839, 390	1*	NA	77.27	–0.081	0.9140

* Model order selected + largest in absolute value.

Source: NCI (1976).

Table G-19. Female B6C3F1 mice—internal dose metric (total oxidative metabolism): BMD and risk estimates (values rounded to 4 significant figures) (inferences for BMR of 0.05 extra risk at 95% confidence level)

	Liver hepatocellular carcinomas	Lung adenomas + carcinomas	Hematopoietic lymphomas + sarcomas
Parameters used in models	q0, q1, q2	q0, q1, q2	q0, q1
p-Value for BMDS model	0.7618	0.7197	0.9140
BMD_{05} (from BMDS)	352.4	517.8	423.8
BMD_{05} (median, mode from WinBUGS)	284.8, 292.5	414.3, 299.9	409.8, 382.6
BMDL (BMDS)*	138.1	193.0	189.5
BMDL (5^{th} percentile, WinBUGS)	162.6	195.4	226.2
BMD_{05} for Combined Risk (median, mode, from WinBUGS)	136.1, 121.1		
BMDL for Combined Risk (5^{th} percentile, WinBUGS)	85.65		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.004123	0.001912	0.0120315
Upper 95% CL	0.04039	0.02919	0.0295375
Sum of risks at dose 100			
WinBUGS Bayes risk estimates			
Risk at dose 100: mean, median	0.01468, 0.01311	0.01284, 0.01226	0.009552, 0.008286
Upper 95% CL	0.03032	0.02590	0.021410
Comb. risk at dose 100 mean, median	0.03663, 0.03572		
Comb. risk at dose 100, upper 95% CL	0.05847		

* All confidence intervals are at 5% (lower) or 95% (upper) level, one-sided.

Source: NCI (1976).

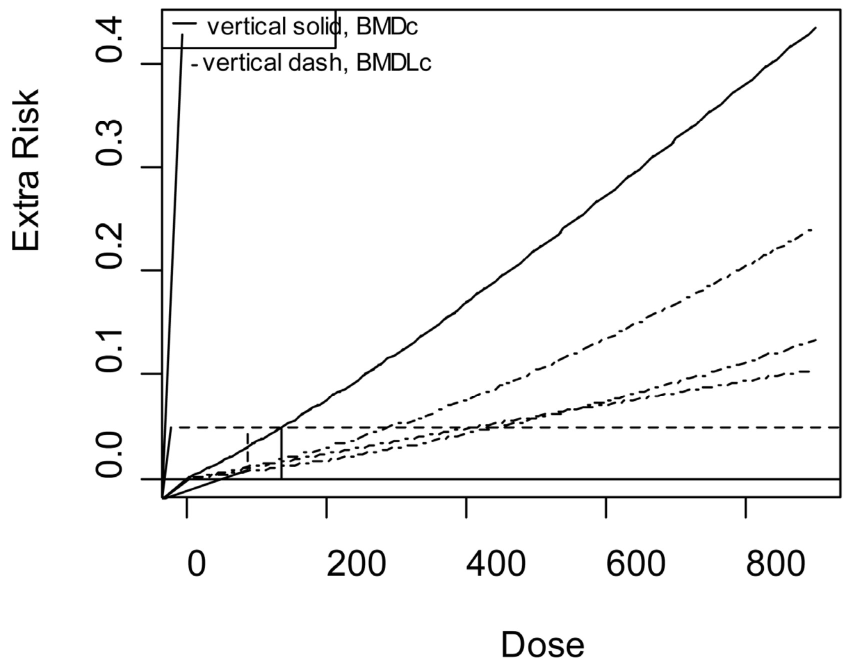

Figure G-7. Female B6C3F1 mice—internal dose metric (total oxidative metabolism): combined and individual tumor extra-risk functions.

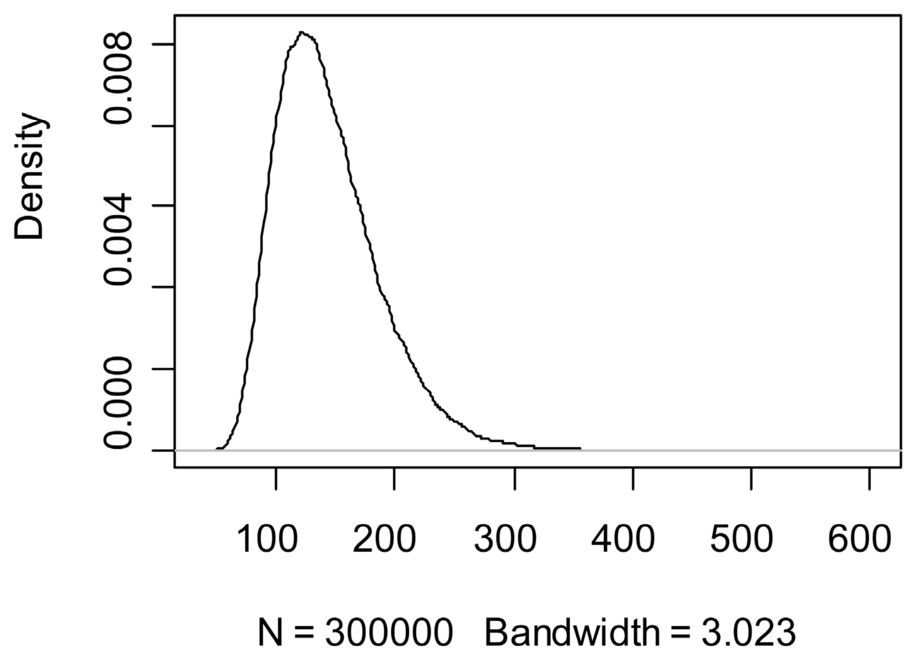

Figure G-8. Female B6C3F1 mice—internal dose metric (total oxidative metabolism): posterior distribution of BMDc for combined risk.

Table G-20. B6C3F1 female mice inhalation exposure—internal dose metric (total oxidative metabolism)

Internal dose[a]	Liver hepatomas/N[b]	Lung adenomas + carcinomas/N[b]
0	3/88	2/90
280.946	4/89	6/90
622.530	4/88	7/89
939.105	9/85	14/87

[a] Internal dose, Total Oxidative Metabolism, adjusted for body weight, units (mg/[wk-kg$^{3/4}$]). Internal doses were adjusted by a factor 0.75, accounting for exposure duration 78/104 weeks. Before adjustment, median internal doses were 374.5945, 830.0405, 1252.14 (mg/[wk-kg$^{3/4}$]).
[b] Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study

Source: Maltoni (1986).

Table G-21. B6C3F1 female mice—internal dose: model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order, *selected	Coeff. estimates equal zero	AIC	Largest+ scaled residual	Goodness of fit p-value
Liver	3*	β1, β2	153.1	–0.410	0.8511
	2	β1	153.4	–0.625	0.7541
	1	NA	154	–0.816	0.5571
Lung	3	β2	195.8	–0.571	0.3995
	2	NA	195.9	–0.671	0.3666
	1*	NA	194	–0.776	0.6325

* Model order selected + largest in absolute value.

Source: Maltoni (1986).

Table G-22. B6C3F1 female mice inhalation exposure—internal dose metric (total oxidative metabolism) (inferences for 0.05 extra risk at 95% confidence level)

	Liver hepatomas	Lung adenomas + carcinomas
Parameters used in models	q0, q1, q2, q3	q0, q1
p-Value for BMDS model	0.5571	0.6325
BMD_{05} (from BMDS)	813.7	366.7
BMD_{05} (median, mode—WinBUGS)	672.9, 648.0	382.8, 372.1
BMDL (BMDS)*	419.7	244.6
ms_combo BMD_{05}c, BMDLc	412.76, 189.23	
BMDL (5^{th} percentile, WinBUGS)	482.7	251.1
BMD_{05} for combined risk (median, mode, from WinBUGS)	286.7, 263.1	
BMDL for combined risk (5^{th} percentile, WinBUGS)	199.5	
BMDS maximum likelihood risk estimates		
Risk at dose 100	0.006284	0.01389
Upper 95% CL	0.01335	0.02215
Sum of risks at dose 100	0.02017	
WinBUGS Bayes risk estimates: means (medians)		
Risk at dose 100: mean, median	0.003482, 0.002906	0.01337, 0.01331
Upper 95% CL,	0.008279	0.02022
Comb. risk at dose 100 mean, median	0.01637, 0.01621	
Comb. risk at dose 100, upper 95% CL	0.02455	

* All confidence intervals are at 5% (lower) or 95% (upper) level, one-sided.

Source: Maltoni (1986).

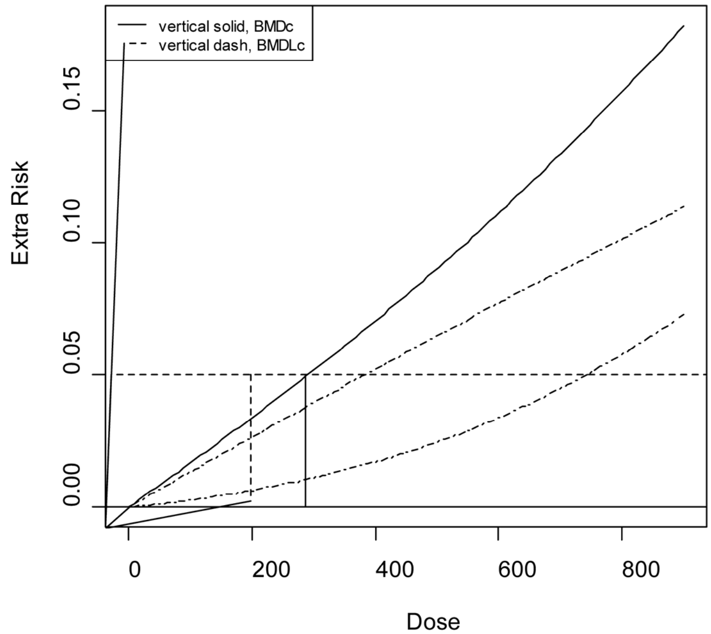

Figure G-9. B6C3F1 female mice inhalation exposure—internal dose metric: combined and individual tumor extra-risk functions.

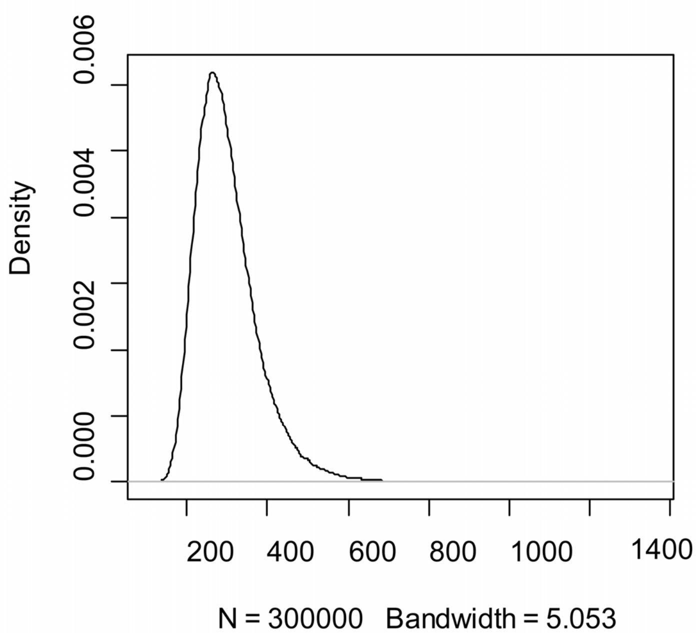

Figure G-10. B6C3F1 female mice inhalation exposure—internal dose metric: posterior distribution of BMDc for combined risk.

Table G-23. Maltoni Sprague-Dawley male rats—internal dose metric (total metabolism)

Internal dose[a]	Kidney adenomas + carcinomas/N^b	Leukemias/N^b	Testis, Leydig cell tumors/N^b
0	0/121	9/134	6/121
214.6540	1/118	13/130	16/116
507.0845	0/116	14/130	30/116
764.4790	5/123	15/129	31/122

[a] Internal dose, Total Oxidative Metabolism, adjusted for body weight, units [mg/(wk-kg$^{3/4}$)].
[b] Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study.

Table G-24. Maltoni Sprague-Dawley male rats—internal dose model selection comparison of model fit statistics for multistage models of increasing order

Tumor site	Model order, *selected	Coeff. estimates equal zero	AIC	Largest* scaled residual	Goodness of fit *p*-value
Kidney	3	γ, β2	61.35	–1.264	0.262
	2	γ	61.75	–1.343	0.246
	1*	γ	60.32	–1.422	0.370
Leukemias	3	β2, β3	336.5	0.479	0.828
	2	β2	336.5	0.479	0.828
	1*	NA	336.5	0.479	0.828
Testis, Leydig cell tumors	3	β2, β3	417.7	1.008	0.363
	2	β2	417.7	1.008	0.363
	1*	NA	417.7	1.008	0.363

* Largest in absolute value.

Table G-25. Maltoni Sprague-Dawley male rats—internal dose metric (total metabolism) (inferences for 0.01 extra risk at 95% confidence level)

	Kidney adenomas + carcinomas	Leukemias	Testis, Leydig cell tumors
Parameters used in models	q0, q1	q0, q1	q0, q1
p-Value for BMDS model	0.3703	0.8285	0.3626
BMD_{01} (from BMDS)	295.1	145.8	26.65
BMD_{01} (median, mode—WinBUGS)			
BMDL (BMDS)*	161.3	65.29	20.32
BMDL (5th percentile, WinBUGS)			
BMD_{01} for combined risk (median, mode, from WinBUGS)	20.97, 19.73		
BMDL for combined risk (5th percentile, WinBUGS)	16.14		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.003400	0.0068694	0.0370162
Upper 95% CL	0.0068784	0.0169134	0.0504547
Sum of risks at dose 100	0.04729		
Risk at dose 10	0.0003406	0.0006891	0.0037648
Upper 95% CL	0.0006900	0.0017044	0.0051638
Sum of risks at dose 10	0.004795		
WinBUGS Bayes risk estimates: means (medians)			
Risk at dose 100: mean, median	0.003191, 0.003028	7.691e-03, 7.351e-03	0.03641, 0.03641
Upper 95% CL	0.006044	1.539e-02	0.04769
Comb. risk at dose 100—mean, median	0.04688, 0.04680		
Comb. risk at dose 100, upper 95% CL	0.060380		
Risk at dose 100—mean, median	3.196e-04, 3.032e04	7.726e-04, 7.376e04	0.003705, 0.003703
Upper 95% CL	6.060000e-04	1.550000e-03	0.004874000
Comb. risk at dose 10—mean, median	0.004793, 0.0047820		
Comb. risk at dose 10, upper 95% CL	0.006208		

* All confidence intervals are at 5% (lower) or 95% (upper) level, one-sided.

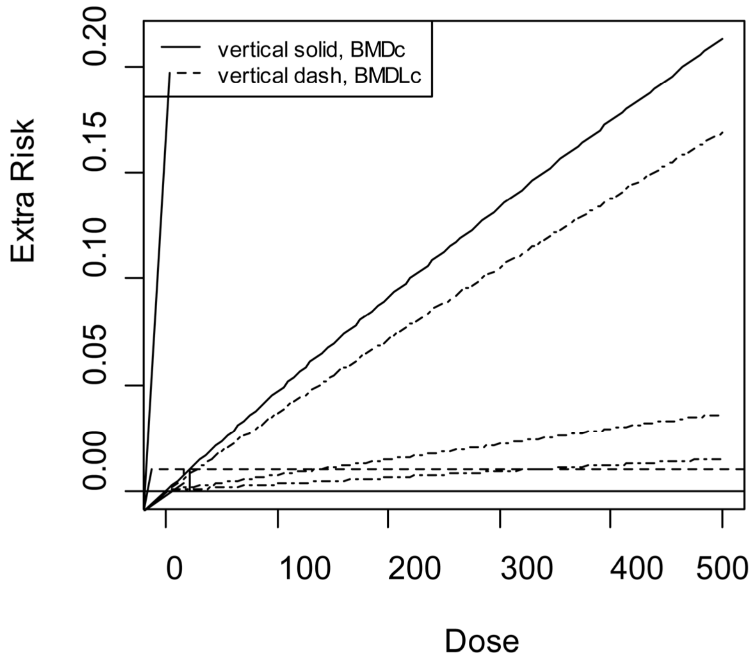

Figure G-11. Maltoni Sprague-Dawley male rats—internal dose metric: combined and individual tumor extra-risk functions.

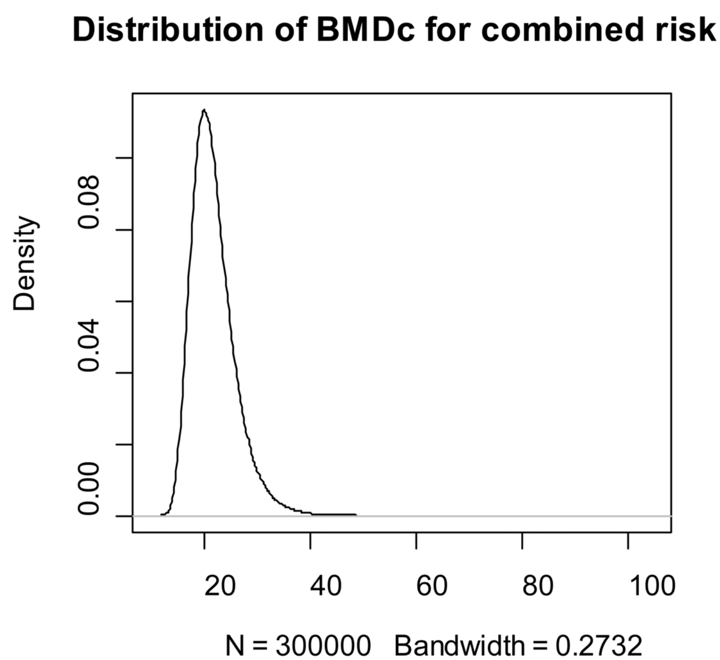

Figure G-12. Maltoni Sprague-Dawley male rats—internal dose metric: posterior distribution of BMDc for combined risk.

G.9. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK)-MODEL UNCERTAINTY ANALYSIS OF UNIT RISK ESTIMATES

As discussed in Section 5.2, an uncertainty analysis was performed on the unit risk estimates derived from rodent bioassays to characterize the impact of pharmacokinetic uncertainty. In particular, two sources of uncertainty are incorporated: (a) uncertainty in the rodent internal doses for each dose group in each chronic bioassay and (b) uncertainty in the relationship between exposure and the human population mean internal dose at low exposure levels.

A Bayesian approach provided the statistical framework for this uncertainty analysis. Rodent bioassay internal dose-response relationships were modeled with the multistage model, with general form

$$P(id) = 1 - \exp[-(q_0 + q_1 id + q_2 id^2 + ... + q_k id^k)], \qquad \text{(Eq. G-9)}$$

where $P(id)$ represents the lifetime risk (probability) of cancer at *internal* dose id, and multistage parameters $q_i \geq 0$, for $i = 0, 1, ..., k$. Since the BMD (in internal dose units) for a given BMR can be derived from the multistage model parameters q_i, it is sufficient to estimate the posterior distribution of q_i given the combined bioassay data (for each dose group j, the number responding y_j, the number at risk n_j, and the administered dose d_j) and the rodent pharmacokinetic data, for which the posterior distribution can be derived using the Bayesian analysis of the PBPK model described in Section 3.5. In particular, the posterior distribution of q_i can be expressed as

$$P(q_{[i]}|D_{bioassay}\ D_{pk}) \propto P(q_{[i]})\ P(y_{[j]}|\ q_{[i]}\ n_{[j]})\ P(id_{[j]}|d_{[j]}, D_{pk}) \qquad \text{(Eq. G-10)}$$

Here, the first term after the proportionality $P(q_{[i]})$ is the prior distribution of the multistage model parameters (assumed to be noninformative), the second term $P(y_{[j]}|q_{[i]}\ n_{[j]})$ is the likelihood of observing the bioassay response given a particular set of multistage parameters and the number at risk (the product of binomial distributions for each dose group), and $P(id_{[j]}|d_{[j]}, D_{pk})$ is the posterior distribution of the rodent internal doses $id[_j]$, given the bioassay doses and the pharmacokinetic data used to estimate the PBPK model parameters.

The distribution of unit risk ($UR_{id} = BMR/BMD$) estimates in units of "per internal dose" is then derived deterministically from the distribution of multistage model parameters:

$$P(UR_{id}|D_{bioassay}\ D_{pk\text{-}rodent}) = \int P(q_{[i]}|D_{bioassay}\ D_{pk\text{-}rodent})\ \delta[UR - BMR/BMD(q_{[i]})]\ dq_{[i]} \quad \text{(Eq. G-11)}$$

Here δ is the Dirac delta-function. Then, the distribution of unit risk estimates in units of "per human exposure" (per mg/kg/d ingested or per continuous ppm exposure) is derived by converting the unit risk estimate in internal dose units:

$$P(UR_{human}|D_{bioassay}\ D_{pk\text{-}rodent}) = \int P(UR_{id}|D_{bioassay}\ D_{pk\text{-}rodent})\ P(id_{conversion}|D_{pk\text{-}human})$$
$$\delta(UR_{human} - UR_{id} \times id_{conversion})\ d\mathrm{id}_{conversion} \qquad \text{(Eq. G-12)}$$

Here, $id_{conversion}$ is the population mean of the ratio between internal dose and administered exposure at low dose (0.001 ppm or 0.001 mg/kg/d), and $P(id_{conversion}|D_{pk\text{-}human})$ is its posterior distribution from the Bayesian analysis of the human PBPK model.

This statistical model was implemented via Monte Carlo as follows. For each bioassay, for a particular iteration r ($r = 1 \ldots n_r$),

(1) A sample of rodent PBPK model *population* parameters $(\mu,\Sigma)_{rodent,r}$ was drawn from the posterior distribution. Using these population parameters, a single set of *group* rodent PBPK model parameters $\theta_{rodent,r}$ was drawn from the population distribution. As discussed in Section 3.5, for rodents, the population model describes the variability among groups of rodents, and the group-level parameters represent the "average" toxicokinetics for that group.

(2) Using $\theta_{rodent,r}$, the rodent PBPK model was run to generate a set of internal doses $id_{[j],r}$ for the bioassay.

(3) Using this set of internal doses $id_{[j],r}$, a sample $q_{[i],r}$ was selected from the distribution (conditional on $id_{[j],r}$) of multistage model parameters, generated using the WinBUGS, following the methodology of Kopylev et al. (2007).

(4) The unit risk in internal dose units $UR_{id,r} = BMR/BMD(q_{[i],r})$ was calculated based on the multistage model parameters.

(5) A sample of human PBPK model *population* parameters $(\mu,\Sigma)_{human,r}$ was drawn from the posterior distribution. Using these population parameters, multiple sets of *individual* human PBPK model parameters $\theta_{human,r,[s]}$ ($s = 1 \ldots n_s$) were generated. A continuous exposure scenario at low exposure was run for each individual, and the population mean internal dose conversion was derived by taking the arithmetic mean of the internal dose conversion for each individual: $id_{conversion,r} = \mathrm{Sum}(id_{conversion,r,s})/n_s$.

(6) The sample for the unit risk in units per human exposure was calculated by multiplying the sample for the unit risk in internal dose units by the sample for the population internal dose conversion: $UR_{human,r} - UR_{id,r} \times id_{conversion,r}$.

In practice, samples for each of the above distributions were "precalculated," and inferences were performed by re-sampling (with replacement) according to the scheme above.

For the results described in Section 5.2, a total of n_r = 15,000 samples was used for deriving summary statistics. For calculating the unit risks in units of internal dose, the BMDs were derived by re-sampling from a total of 4.5×10^6 multistage model parameter values (1,500 rodent PBPK model parameters from the Bayesian analysis described in Section 3.5, for each of which there were conditional distributions of multistage model parameters of length 3,000 derived using WinBUGS). The conversion to unit risks in units of human exposure was re-sampled from 500 population mean values, each of which was estimated from 500 sampled individuals.

The file Appendix.linked.files\AppG.Cancer.Rodents.Uncertainty.Analysis.TCE.DRAFT.pdf contains summary statistics (mean, and selected quantiles from 0.01 to 0.99) from these analyses, and is the source for the results presented in Chapter 5 (see Tables 5-34 and 5-35). Histograms of the distribution of unit risks in per unit human exposure are in the file Appendix.linked.files\AppG.Cancer.Rodents.uncertainty.CSF-inhal.histograms.inhalation.bioassays.TCE.DRAFT.pdf for the rodent inhalation bioassays and Appendix.linked.files\AppG.Cancer.Rodents.uncertainty.CSF-oral.histograms.oral.bioassays.TCE.DRAFT.pdf for the rodent oral bioassays. Route-to-route extrapolated unit risks are in the files Appendix.linked.files\AppG.Cancer.Rodents.uncertainty.CSF-inhal.histograms.oral.bioassays.TCE.DRAFT.pdf (inhalation unit risks extrapolated from oral bioassays) and Appendix.linked.files\AppG.Cancer.Rodents.uncertainty.CSF-oral.histograms.inhalation.bioassays.TCE.DRAFT.pdf (oral unit risks extrapolated from inhalation bioassays). Each figure shows the uncertainty distribution for the male and female combined population risk per unit exposure (transformed to base-10 logarithm), with the exception of testicular tumors, for which only the population risk per unit exposure for males is shown.

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APPENDIX H

Lifetable Analysis and Weighted Linear Regression based on Results from Charbotel et al. (2006)

CONTENTS—Appendix H: Lifetable Analysis and Weighted Linear Regression based on Results from Charbotel et al. (2006)

APPENDIX H: LIFETABLE ANALYSIS AND WEIGHTED LINEAR REGRESSION BASED ON RESULTS FROM CHARBOTEL ET AL. (2006)

H.1. LIFETABLE ANALYSIS

A spreadsheet illustrating the extra-risk calculation for the derivation of the lower 95% bound on the effective concentration associated with a 1% extra risk (LEC_{01}) for renal cell carcinoma (RCC) incidence is presented in Table H-1.

H.2. EQUATIONS USED FOR WEIGHTED LINEAR REGRESSION OF RESULTS FROM CHARBOTEL ET AL. (2006) (source: Rothman [1986], p. 343-344)

Linear model: RR = 1 + bX

where RR = risk ratio, X = exposure, and b = slope

b can be estimated from the following equation:

$$\hat{b} = \frac{\sum_{j=2}^{n} w_j x_j \hat{RR}_j - \sum_{j=2}^{n} w_j x_j}{\sum_{j=2}^{n} w_j x_j^2} \quad \text{(Eq. H-1)}$$

where j specifies the exposure category level and the reference category (j = 1) is ignored.

The standard error of the slope can be estimated as follows:

$$SE(\hat{b}) \approx \sqrt{\frac{1}{\sum_{j=2}^{n} w_j x_j^2}} \quad . \quad \text{(Eq. H-2)}$$

The weights, w_j, are estimated from the confidence intervals (as the inverse of the variance):

$$Var(\hat{RR}_j) \approx \hat{RR}_j^2 Var[\ln(\hat{RR}_j)] \approx \hat{RR}_j^2 \times \left[\frac{\ln(\overline{RR}_j) - \ln(\underline{RR}_j)}{2 \times 1.96}\right]^2 \quad \text{(Eq. H-3)}$$

where $\overline{RR}_j$ is the 95% upper bound on the RR_j estimate (for the jth exposure category) and $\underline{RR}_j$ is the 95% lower bound on the RR_j estimate.

Table H-1. Extra-risk calculation[a] for environmental exposure to 1.82 ppm TCE (the LEC_{01} for RCC incidence)[b] using a linear exposure-response model based on the categorical cumulative exposure results of Charbotel et al. (2006), as described in Section 5.2.2.1.2.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (*i*)	**Age interval**	**All cause mortality (×10⁵/yr)**	**RCC incidence (×10⁵/yr)**	**All cause hazard rate (*h**)**	**Prob. of surviving interval (*q*)**	**Prob. of surviving up to interval (*S*)**	**RCC cancer hazard rate (*h*)**	**Cond. prob. of RCC incidence in interval (*Ro*)**	**Exp. duration mid interval (*x*time)**	**Cum. exp. mid interval (*x*dose)**	**Exposed RCC hazard rate (*hx*)**	**Exposed all cause hazard rate (*h*x*)**	**Exposed prob. of surviving interval (*qx*)**	**Exposed prob. of surviving up to interval (*Sx*)**	**Exposed cond. prob. of RCC in interval (*Rx*)**
1	<1	685.2	0	0.0069	0.9932	1.0000	0.000000	0.000000	0.5	2.77	0.000000	0.0069	0.9932	1.0000	0.000000
2	1-4	29.9	0	0.0012	0.9988	0.9932	0.000000	0.000000	3	16.61	0.000000	0.0012	0.9988	0.9932	0.000000
3	5-9	14.7	0	0.0007	0.9993	0.9920	0.000000	0.000000	7.5	41.52	0.000000	0.0007	0.9993	0.9920	0.000000
4	10-14	18.7	0.1	0.0009	0.9991	0.9913	0.000005	0.000005	12.5	69.20	0.000006	0.0009	0.9991	0.9913	0.000006
5	15-19	66.1	0.1	0.0033	0.9967	0.9903	0.000005	0.000005	17.5	96.88	0.000006	0.0033	0.9967	0.9903	0.000006
6	20-24	94	0.2	0.0047	0.9953	0.9871	0.000010	0.000010	22.5	124.56	0.000013	0.0047	0.9953	0.9871	0.000013
7	25-29	96	0.7	0.0048	0.9952	0.9824	0.000035	0.000034	27.5	152.24	0.000049	0.0048	0.9952	0.9824	0.000048
8	30-34	107.9	1.6	0.0054	0.9946	0.9777	0.000080	0.000078	32.5	179.91	0.000117	0.0054	0.9946	0.9777	0.000114
9	35-39	151.7	3.2	0.0076	0.9924	0.9725	0.000160	0.000155	37.5	207.59	0.000245	0.0077	0.9924	0.9724	0.000237
10	40-44	231.7	6.3	0.0116	0.9885	0.9651	0.000315	0.000302	42.5	235.27	0.000504	0.0118	0.9883	0.9650	0.000484
11	45-49	352.3	11	0.0176	0.9825	0.9540	0.000550	0.000520	47.5	262.95	0.000919	0.0180	0.9822	0.9537	0.000869
12	50-54	511.7	17.3	0.0256	0.9747	0.9373	0.000865	0.000801	52.5	290.63	0.001507	0.0262	0.9741	0.9367	0.001393
13	55-59	734.8	26.2	0.0367	0.9639	0.9137	0.001310	0.001175	57.5	318.31	0.002375	0.0378	0.9629	0.9124	0.002127
14	60-64	1140.1	36.2	0.0570	0.9446	0.8807	0.001810	0.001549	62.5	345.99	0.003409	0.0586	0.9431	0.8786	0.002909
15	65-69	1727.4	44.6	0.0864	0.9173	0.8319	0.002230	0.001777	67.5	373.67	0.004358	0.0885	0.9153	0.8286	0.003456
16	70-74	2676.4	49	0.1338	0.8747	0.7631	0.002450	0.001750	72.5	401.35	0.004961	0.1363	0.8726	0.7584	0.003518
17	75-59	4193.2	51.6	0.2097	0.8109	0.6675	0.002580	0.001554	77.5	429.03	0.005407	0.2125	0.8086	0.6617	0.003223
18	80-84	6717.2	44.4	0.3359	0.7147	0.5412	0.002220	0.001021	82.5	456.71	0.004809	0.3384	0.7129	0.5351	0.002183
							***Ro* =**	0.010736						***Rx* =**	0.020586
Extra risk = (*Rx* − *Ro*)/(1 − *Ro*) = 0.00996															

Column A: interval index number (i).
Column B: 5-year age interval (except <1 and 1−4) up to age 85.
Column C: all-cause mortality rate for interval i ($\times 10^5$/year) (2004 data from NCHS [2007]).
Column D: RCC incidence rate for interval i ($\times 10^5$/year) (2001-2005 SEER data [http://seer.cancer.gov]).
Column E: all-cause hazard rate for interval i (h^*_i) [= all-cause mortality rate × number of years in age interval].[c]
Column F: probability of surviving interval i without being diagnosed with RCC (q_i) [= $\exp(-h^*_i)$].
Column G: probability of surviving up to interval i without having been diagnosed with RCC (S_i) [$S_1 = 1$; $S_i = S_{i-1} \times q_{i-1}$, for i > 1].
Column H: RCC incidence hazard rate for interval i (h_i) [= RCC incidence rate × number of years in interval].
Column I: conditional probability of being diagnosed with RCC in interval i [= $(h_i/h^*_i) \times S_i \times (1-q_i)$], i.e., conditional upon surviving up to interval i without having been diagnosed with RCC [Ro, the background lifetime probability of being diagnosed with RCC = the sum of the conditional probabilities across the intervals].
Column J: exposure duration (in years) at mid-interval ($x$time).
Column K: cumulative exposure mid-interval ($x$dose) [= exposure level (i.e., 1.82 ppm) × 365/240 × 20/10 × $x$time] (365/240 × 20/10 converts continuous environmental exposures to corresponding occupational exposures).
Column L: RCC incidence hazard rate in exposed people for interval i (hx_i) [= $h_i \times (1 + \beta \times x\text{dose})$, where $\beta = 0.001205 + (1.645 \times 0.0008195) = 0.002554$] [0.001205 per ppm × year is the regression coefficient obtained from the weighted linear regression of the categorical results (see Section 5.2.2.1.2). To estimate the LEC_{01}, i.e., the 95% lower bound on the continuous exposure giving an extra risk of 1%, the 95% upper bound on the regression coefficient is used, i.e., MLE + 1.645 × SE].
Column M: all-cause hazard rate in exposed people for interval i (h^*x_i) [= $h^*_i + (hx_i - h_i)$].
Column N: probability of surviving interval i without being diagnosed with RCC for exposed people (qx_i) [= $\exp(-h^*x_i)$].
Column O: probability of surviving up to interval i without having been diagnosed with RCC for exposed people (Sx_i) [$Sx_1 = 1$; $Sx_i = Sx_{i-1} \times qx_{i-1}$, for i> 1].
Column P: conditional probability of being diagnosed with RCC in interval i for exposed people [= $(hx_i/h^*x_i) \times Sx_i \times (1-qx_i)$] (Rx, the lifetime probability of being diagnosed with RCC for exposed people = the sum of the conditional probabilities across the intervals).

[a] Using the methodology of BEIR IV (1988).
[b] The estimated 95% lower bound on the continuous exposure level of TCE that gives a 1% extra lifetime risk of RCC.
[c] For the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval, i.e., (the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval—the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) × number of years in interval. This adjustment was ignored here because the RCC incidence rates are small compared with the all-cause mortality rates.

MLE = maximum likelihood estimate, SE = standard error.

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