G. Appendix: TCE Cancer Dose-Response Analyses with Rodent Cancer Bioassay Data

G.1. Data Sources

TCE cancer endpoints were identified in Maltoni et al. (1986), NCI (1976), 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. We tabulated all endpoint data identified by authors as having a statistically significant response to dose, and we also reviewed data that had marginally significant trends with dose. For all endpoints for which we present dose-response model estimates, we verified trends 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, we used the number alive at 52 weeks (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 from 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 we used an alternative number at risk 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), we used the reported "effective number of mice" in their Table 2, 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), we used 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).

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), we used the number of mice with tumors observed by week 104 (their Figure 2). We used the cumulative incidence reported by Fukuda et al. (1983) at 107 weeks (after 104 weeks of exposure). For the

NCI (1976) and NTP (1988, 1990) studies, we used the reported cumulative incidence at 103 to 107 weeks (study time varied by study and species).

G.2. Internal Dose Metrics and Dose Adjustments

PBPK modeling was used to estimate levels of dose metrics corresponding to different exposure scenarios in rodents and humans (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 the table below.

Table G.2.1 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, we used central estimates specific to each species, strain, and sex (and sub-study). 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, we concluded that a plausible range for lifetime average body weight is 20 – 35 gm, with a median near 28 gm. For these two studies, we computed internal dose metrics for these three average body weights (20, 28, and 35 gm). We then evaluated the percentage differences between the internal dose metrics for the intermediate body weight (BW) of 28 gm and the low and high average BW of 20 gm and 35 gm. Internal

[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,

dose metrics were little affected by choice of body weight. For all dose metrics, the differences were less than ±13%.

The medians (from the MCMC 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 non-linear. We are interested in estimating the expected response. The expected value of a nonlinear function of dose is under- or over-estimated 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)] <= E[f(X)]). For the dose-response function, we are 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, we compared results based on the posterior median and the posterior mean dose metric 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) × D1 + (66/78) × 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 (at 91 weeks rather than 104 weeks) by a factor of (91 wk / 104 wk)^3.[2]

G.4. Rodent to Human Dose Extrapolation

Adjustments for rodent-to-human extrapolation were applied to the final results – the BMD, BMDL, and cancer slope factor (potency), which is calculated as BMR/BMDL, e.g., $0.10/BMDL_{10}$.

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 EPA 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 $(BW_{Human} / BW_{Animal})^{\wedge}0.75$. To extrapolate animal inhalation exposure to human oral exposure, we used the following equation[3]:

Animal, equivalent oral intake, mg/kg/day =

ppm * [MW_{TCE} / 24.45 [4]] * MV * (60 min/hr) * (10^3 mg/g) * [24hr / BW_{kg}]

with units: ppm * [g/mol ÷ L/mol] * L/min * (min/hr) * (mg/g) * [hr/day ÷ kg]

[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 3 (i.e., a factor [length of study in wks/104 wks]^3) to reflect the fact that the animals were not observed for the full standard lifetime (U.S. EPA, 1980).

[3] ToxRisk version 5.3, © 2000-2001 by the KS Crump Group, Inc.

[4] Molecular weight of TCE is 131.39; there are 24.45 liters of perfect gas per g-mol at standard temperature & pressure, USEPA (1994).

which reduces to ppm * [7.738307 * MV / BW.kg]

where

ppm = Animal inhalation concentration, $1/10^6$, unitless

MV = minute volume (breathing rate) at rest, liters / minute.

Minute volume (MV) was estimated using equations from U.S. EPA (1994, p.4-27),

Mouse: $\ln(MV) = 0.326 + 1.05 * \ln(BW_{kg})$

Rat: $\ln(MV) = -0.578 + 0.821 * \ln(BW_{kg})$.

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[5].

To extrapolate animal oral exposure to equivalent human inhalation exposure, we reversed the calculation above 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 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. We therefore used only experiment BT306bis males in our 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*

[5] Find whole animal intake from mg/kg-day * 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-day for the human. The net effect is Animal mg/kg-day * (BW_{Animal} / BW_{Human})^0.25 = Human mg/kg-day.

of the same breed, divided by litter distribution within the two experiments, they have been evaluated separately and comprehensively." We also analyzed the data separately and in combination.

The data and modeling results for these tumors in the BT304 and BT304bis experiments is tabulated below. We 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.

1

Table G.5.1. 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 [1]

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 [2]				
				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 & 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					

[1] First tumor occurrences were not reported separately by sex

[2] Models of orders 3 were fitted; the highest-order non-zero 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).

2

1

Table G.5.2. 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 [1]

Exposure Concen. (ppm)	No. alive	No. rats with this cancer	Proportion with cancer	Multistage model fit statistics [2]				
				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 & 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					

[1] First tumor occurrences were not reported separately by sex

[2] Models of orders 3 were fitted; the highest-order non-zero 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.

2

1

Table G.5.3. 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 [1]

Exposure Concen. (ppm)	No. alive	No. rats with this cancer	Proportion with cancer	Multistage model fit statistics [2]				
				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 & 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					

[1] First tumor occurrences were not reported separately by sex

[2] Models of orders 3 were fitted; the highest-order non-zero 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.

2

1

Table G.5.4. 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[1].

Exposure Concen. (ppm)	No. alive	No. rats with this cancer	Proportion with cancer	Multistage model fit statistics [2]				
				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 & 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					

[1] Numbers alive reported for weeks as close as possible to week 52 (first tumors observed at weeks 81, 62, respectively, for the two experiments).
[2] Models of orders 3 were fitted; the highest-order non-zero 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.

2

G.6. Dose-Response Modeling Results

Using BMDS software, we fitted the multistage quantal model 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, we then also fitted lower-order models, down to a 1st-order multistage model. This permitted us to screen results efficiently.

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[6], 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, 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 numbers "GRP" are arbitrary, and are the same as GRP

[6] 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.

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.

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. We identified three such studies, all with male rats (see Table 5.2.3). Using both survival-adjustment approaches, BMDs and BMDLs were obtained and unit risks derived. A comparison of the results for the three datasets and for various dose metrics is presented in Section 5.2.1.3.

G.7.1. Time-to-tumor modeling

The first approach we 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

$$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) * (t - t_0)^z],$$

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 non-fatal 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 (esp., toxic effects) operating in these studies (also note that cause of death was not reported by the studies we 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 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. (EPA and Battelle confirmed that MSW gave results essentially identical to those of "ToxRisk", a program no longer commercially issued or supported). EPA's BMDS web site provided reviewers' comments and EPA's responses.[7] The MSW program and reports on statistical & computational methodology and model testing will be made available in mid 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 POD 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 EPA's BMDS multistage model, and multistage model selection was conducted as described in Section 5.2.

[7] At http://www.epa.gov/ncea/bmds/response.html under title "2007 External Review of New Quantal Models"; use links to comments and responses.

A detailed exposition is given by Piegorsch and Bailer (1997), Chapter 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. We assumed the 'default' power of 3 (thus, "poly-3"), which was found to be representative for a large number of cancer types (Portier et al., 1986). Algebraically,

$N_{adj} = \sum_i w_i$ where $w_i = 1$ if tumor is present and

$w_i = (t_i / T)^3$ if tumor is absent at time of death (t_i),

and T = duration of study. N was rounded to the nearest integer.[8]

Calculations are reproduced in the spreadsheets 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 5.2.3, 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 an MLE (BMD) for the combined risk. However, the confidence bounds for the combined risk estimate are not calculated by available modeling software. Therefore, we used a Bayesian approach to estimate confidence bounds on the combined BMD. This approach was implemented using the freely available WinBugs software (Spiegelhalter et al., 2003), which

[8] 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 (lower confidence limit of BMD) may be somewhat lower than could be obtained by more laborious calculations.

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 software. These were multistage models with coefficients constrained to be non-negative. The order of model fitted was g-1, where g is the number of dose groups. For internal dose metrics, we used the values shown in tables above.

The multistage model was modified for 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, we were able to report confidence intervals for extra risk in addition to BMDs.

G.8.1.2. *Combined risk from multiple tumor sites*

The multistage model identified by BMDS (example: gamma = 0, beta.1 > 0, beta.2 = 0, beta.3 >0) was used in a WinBUGs script to generate posterior distributions for model parameters, the benchmark dose (BMD) and extra risk at the same dose specified for the BMDS estimates. We used a burn-in of length 10,000, then 100,000 updates, and thinned the latter to every 10th update for sample monitoring. From a WinBUGs run, we archived the sample histories, posterior distribution plots, summary statistics, and codas.

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. We also computed BMD and extra risk for the combined risk function (assuming independence) following Bogen.[9] Results were summarized as percentiles, means and modes (of the smoothed posterior distributions). We also summed the extra risks across tumor types at a specific dose (we used 10 or 100).

BMDLs for rodent internal doses, reported below, were converted to human external doses using the conversion factors in the following Tables (based on PBPK model described in Section 3.5).

Table G.8.1

Rodent to human conversions for internal dose metric TotOxMetabBW34

[9] 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]

Route	sex	human (mean)
Inhal, ppm	F	9.843477
	M	9.702822
Oral, mg/kg-day	F	15.72291
	M	16.4192

Table G.8.2

Rodent to human conversions for internal dose metric TotMetabBW34

route	sex	human (mean)
Inhal, ppm	F	11.84204
	M	11.69996
Oral, mg/kg-day	F	18.76327
	M	19.6

Application of rodent to human conversion factors:

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

G.8.2. Results

Results follow in this order:

Applied doses

NCI, 1976, Female B6C3F1 mice, oral gavage, Liver & Lung tumors and Lymphomas

Maltoni 1986 Female B6C3F1 mice, inhalation (expt. BT306), Liver & Lung tumors

Maltoni 1986 Male Sprague-Dawley rats, inhalation (expt. BT304), Kidney tumors, Testis Leydig Cell tumors, and Lymphomas

Internal Doses

NCI, 1976, Female B6C3F1 mice, oral gavage, Liver & Lung tumors and Lymphomas

Maltoni 1986 Female B6C3F1 mice, inhalation (expt. BT306), Liver & Lung tumors

Maltoni 1986 Male Sprague-Dawley rats, inhalation (expt. BT304), Kidney tumors, Testis Leydig Cell tumors, and Lymphomas

1

NCI, 1976: Female B6C3F1 mice – applied doses : DATA				
Dose*	N **	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
* Doses 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-day, high-dose 1739 mg/kg-day. ** Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study				

2

NCI, 1976: 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	β2	77.28	0.113	0.8812
	1*	NA	77.28	0.113	0.8812
* largest in absolute value					

3

1

NCI, 1976: 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
BMD05 (from BMDS)	138.4	295.2	358.8
BMD05 (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}%-ile, WinBugs)	97.48	150.7	157.7
BMD05 for Combined Risk (median, mode, from WinBugs)	84.99, 78.95		
BMDL for Combined Risk (5_{th}%-ile, WinBugs)	53.61		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.03640	0.01722	0.01419
Upper 95% C.L.	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% C.L.	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			

2

3

Figure G.8.1. Combined and Individual Tumor Extra Risk Functions

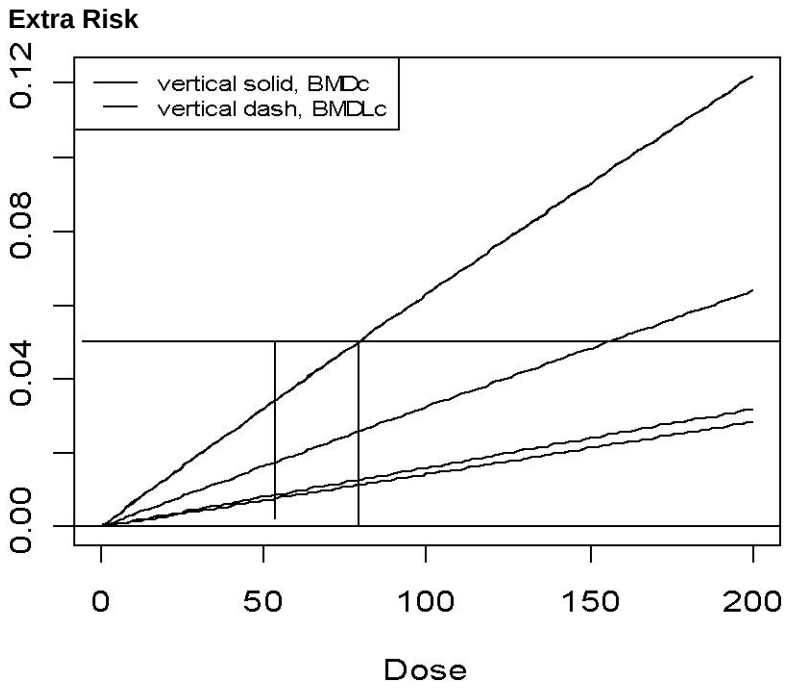
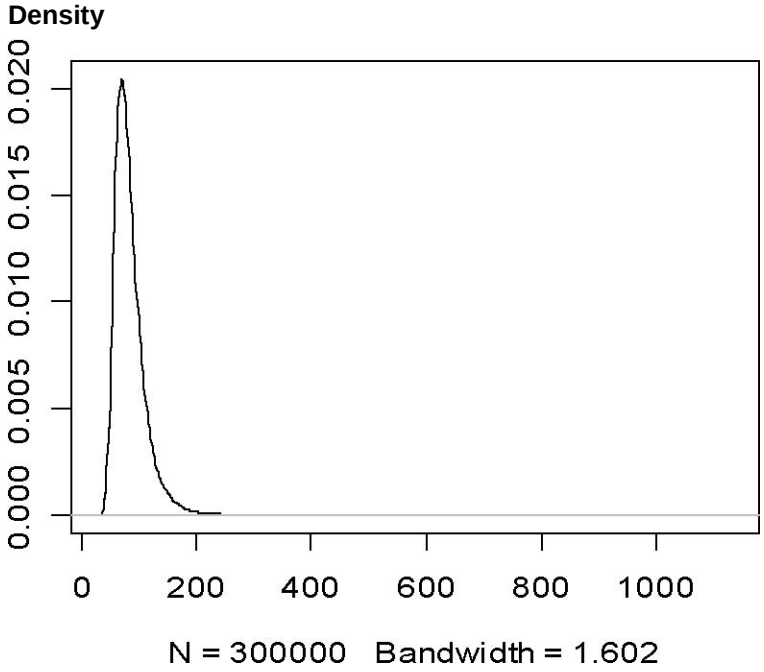


Figure G.8.2. Distribution of BMDc for combined risk



1

Maltoni 1986 B6C3F1 female mice inhalation expos – applied doses				
Dose*		Liver hepatomas / N**	Lung adenomas + carcinomas / N**	
0		3 / 88	2 / 90	
15.6		4 / 89	6 / 90	
46.9		4 / 88	7 / 89	
93.8		9 / 85	14 / 87	
* Doses adjusted by a factor 0.133928571, accounting for exposure 7/24 hours/day x 5/7 days/week, and exposure duration 78/104 weeks. Applied doses were 100, 300, and 600 ppm. ** Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study				

2

Maltoni 1986 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					

3

1

Maltoni 1986 B6C3F1 female mice inhalation exposure – applied doses (inferences for 0.05 extra risk at 95% level)			
	Liver hepatomas	Lung adenomas + carcinomas	
Parameters used in model	q0, q1	q0, q1	
P-value for BMDS model	0.7223	.06471	
BMD05 (from BMDS)	72.73	33.81	
BMD05 (median, mode - WinBugs)	71.55, 56.79	34.49, 31.65	
BMDL (BMDS)*	37.13	21.73	
ms_combo.exe BMD05c, BMDLc	32.12, 16.22		
BMD05 (5_{th} %-ile, WinBugs)	37.03	22.07	
BMD05 for Combined Risk (median, mode, from WinBugs)	23.07, 20.39		
BMDL for Combined Risk (5_{th} %-ile, WinBugs)	15.67		
BMDS maximum likelihood risk estimates			
Risk at dose 10	0.0070281	0.0150572	
Upper 95% C.L.	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% C.L.	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			

2

Figure G.8.3. Combined and Individual Tumor Extra Risk Functions

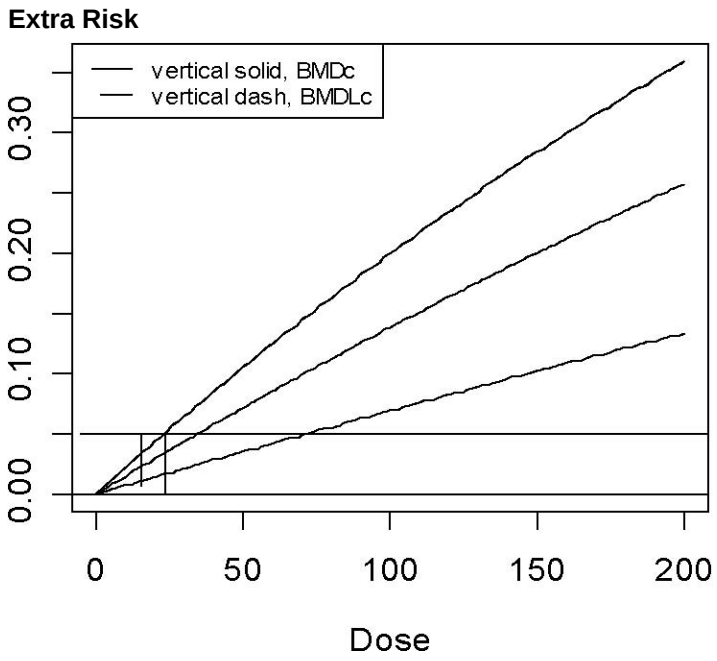
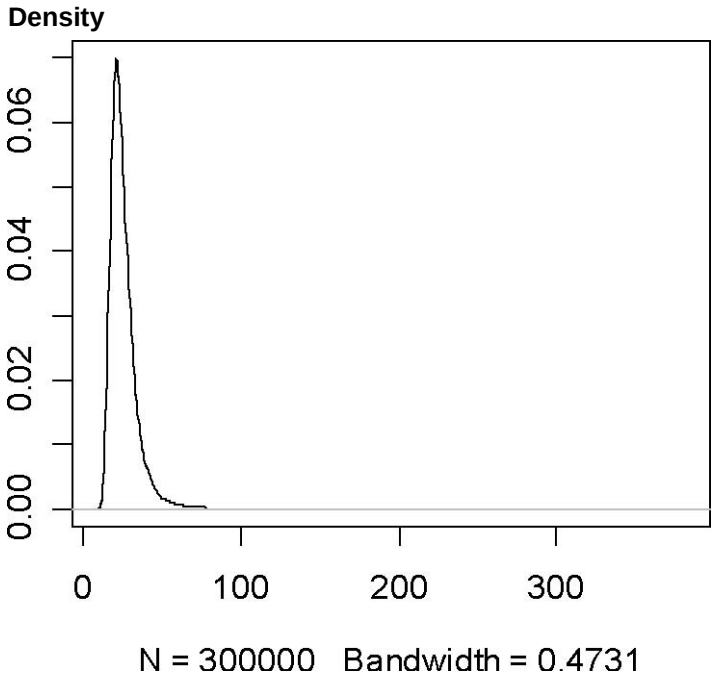


Figure G.8.4. Distribution of BMDc for combined risk



1

Maltoni Sprague-Dawley Male Rats – applied doses				
Dose*		Kidney adenenomas + carcinomas / N**	Leukemias / N**	Testis, Leydig Cell Tumors / N**
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
* Doses adjusted by a factor 0.208333333, accounting for for exposure 7 hours/day x 5/7 days/week. Applied doses were 100, 300, and 600 ppm. ** Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study				

2

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					

3

Maltoni Sprague-Dawley Male Rats – applied doses			
	Kidney adenenomas + 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
BMD01 (from BMDS)	41.47	14.5854	2.46989
BMD01 (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} %-ile, WinBugs)	23.23	5.362	1.789
BMD01 for Combined Risk (median, mode, from WinBugs)	1.960, 1.826		
BMDL for Combined Risk (5_{th} %-ile, WinBugs)	1.437		
BMDS maximum likelihood risk estimates			
Risk at dose 10	0.0024208	0.0068670	0.0398747
Upper 95% C.L.	0.0048995	0.0202747	0.0641010
Sum of risks at dose 10			
Risk at dose 1	0.0002423	0.0006888	0.0040609
Upper 95% C.L.	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% C.L.	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% C.L.	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			

1

Figure G.8.5. Combined and Individual Tumor Extra Risk Functions

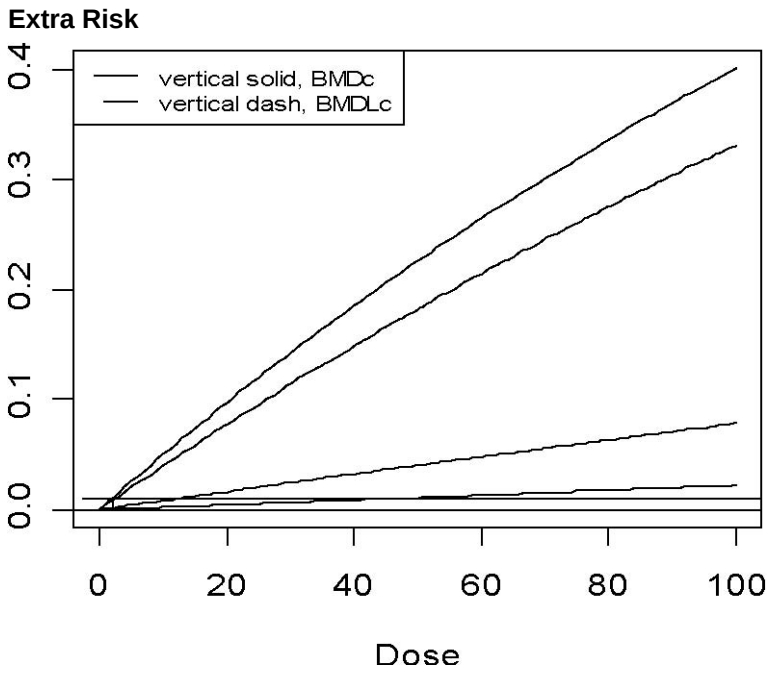
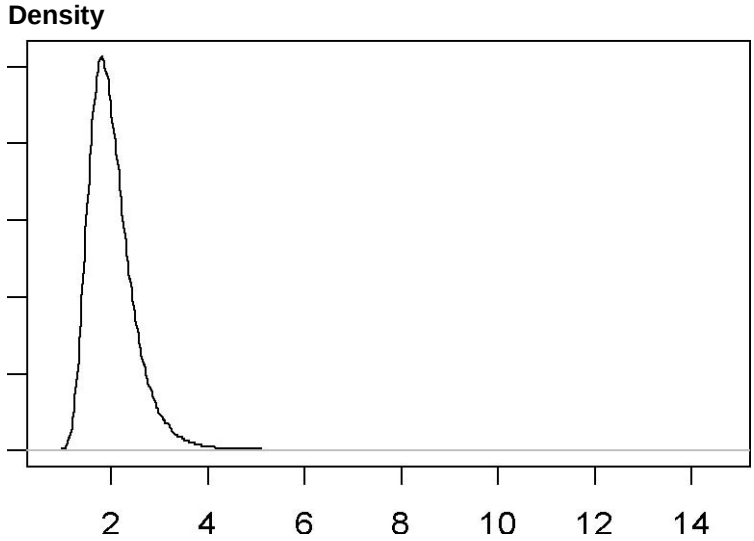


Figure G.8.6. Distribution of BMDc for combined risk



N = 300000 Bandwidth = 0.03059

1

NCI, 1976: Female B6C3F1 mice – Internal Dose Metric (Total Oxidative Metabolism) : DATA				
Internal Dose*	N **	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
* 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). ** Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study				

2

NCI, 1976: 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						

3

4

NCI, 1976: 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
BMD05 (from BMDS)	352.4	517.8	423.8
BMD05 (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} %-ile, WinBugs)	162.6	195.4	226.2
BMD05 for Combined Risk (median, mode, from WinBugs)	136.1, 121.1		
BMDL for Combined Risk (5_{th} %-ile, WinBugs)	85.65		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.004123	0.001912	0.0120315
Upper 95% C.L.	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% C.L.	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			

1

Maltoni 1986 B6C3F1 female mice inhalation expos – Internal Dose Metric (Total Oxidative Metabolism)				
Intern al Dose*		Liver hepatomas / N**	Lung adenomas + carcinomas / N**	
0		3 / 88	2 / 90	
280.946		4 / 89	6 / 90	
622.530		4 / 88	7 / 89	
939.105		9 / 85	14 / 87	
* 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)). ** Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study				

2

Maltoni 1986 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					

3

1

Maltoni 1986 B6C3F1 female mice inhalation expos – Internal Dose Metric (Total Oxidative Metabolism) (inferences for 0.05 extra risk at 95% 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	
BMD05 (from BMDS)	813.7	366.7	
BMD05 (median, mode - WinBugs)	672.9, 648.0	382.8, 372.1	
BMDL (BMDS)*	419.7	244.6	
ms_combo BMD05c, BMDLc	412.76, 189.23		
BMDL (5th %-ile, WinBugs)	482.7	251.1	
BMD05 for Combined Risk (median, mode, from WinBugs)	286.7, 263.1		
BMDL for Combined Risk (5th %-ile, WinBugs)	199.5		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.006284	0.01389	
Upper 95% C.L.	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, 0.00 2906	0.01337, 0.01331	
Upper 95% C.L.,	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			

2

1

Maltoni Sprague-Dawley Male Rats – Internal Dose Metric (Total Metabolism)				
Internal Dose*		Kidney adenenomas + carcinomas / N**	Leukemias / N**	Testis, Leydig Cell Tumors / N**
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
* Internal dose, Total Oxidative Metabolism, adjusted for body weight, units (mg/(wk-kg^3/4)). ** Numbers at risk are the smaller of (a) time of first tumor observation or (b) 52 weeks on study				

2

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					

3

1

Maltoni Sprague-Dawley Male Rats – Internal Dose Metric (Total Metabolism) (inferences for 0.01 extra risk at 95% 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
BMD01 (from BMDS)	295.1	145.8	26.65
BMD01 (median, mode - WinBugs)			
BMDL (BMDS)*	161.3	65.29	20.32
BMDL (5_{th} %-ile, WinBugs)			
BMD01 for Combined Risk (median, mode, from WinBugs)	20.97, 19.73		
BMDL for Combined Risk (5_{th} %-ile, WinBugs)	16.14		
BMDS maximum likelihood risk estimates			
Risk at dose 100	0.003400	0.0068694	0.0370162
Upper 95% C.L.	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% C.L.	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% C.L.	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% C.L.	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			

2

G.9. PBPK-model uncertainty analysis of unit risk estimates

As discussed in Chapter 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: (i) uncertainty in the rodent internal doses for each dose group in each chronic bioassay and (ii) 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

$$P(id) = 1 - \exp[-(q_0 + q_1 id + q_2 id^2 + ... + q_k id^k)],$$

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:

$$P(q_{[i]}|D_{bioassay}\, D_{pk}) \propto P(q_{[i]})\, P(y_{[j]}|\, q_{[i]}\, n_{[j]})\, P(id_{[j]}|d_{[j]}, D_{pk})$$

Here, the first term after the proportionality $P(q_{[i]})$ is the prior distribution of the multistage model parameters (assumed to be non-informative), 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:

$$P(UR_{id}|D_{bioassay}\ D_{pk\text{-}rodent}) = \int P(q_{[i]}|D_{bioassay}\ D_{pk\text{-}rodent})\ \delta(UR\ \ BMR/BMD(q_{[i]}))\ dq_{[i]}$$

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:

$$P(UR_{human}|D_{bioassay}\ D_{pk\text{-}rodent}) = \int P(UR_{id}|D_{bioassay}\ D_{pk\text{-}rodent})\ P(id_{conversion}|D_{pk\text{-}human})\ \delta(UR_{human}\ \ UR_{id} \times id_{conversion})\ did_{conversion}$$

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…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. (2009).

(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…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: $id_{conversion,r} = Sum(\ id_{conversion,r,s}\ )/n_s$.

(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 was "pre-calculated," and inferences were performed by re-sampling (with replacement) according to the scheme above. For the results described in Chapter 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 derived by re-sampling from a total of 4.5×10^6 multistage model parameter values (1500 rodent PBPK model parameters from the Bayesian analysis described in Chapter 3.5, for each of which there were conditional distributions of multistage model parameters of length 3000 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 (Tables 5.2.10 and 5.2.11). 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.

G.10. References

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