4.6. IMMUNOTOXICITY AND CANCERS OF THE IMMUNE SYSTEM

Chemical exposures may result in a variety of adverse immune-related effects, including immunosuppression (decreased host resistance), autoimmunity, and allergy-hypersensitivity, and may result in specific diseases such as infections, systemic or organ-specific autoimmune diseases, or asthma. Measures of immune function (e.g., T-cell counts, immunoglobulin (Ig) E levels, specific autoantibodies, cytokine levels) may provide evidence of an altered immune response that precedes the development of clinically expressed diseases. The first section of this chapter discusses effects relating to immunotoxicity, including risk of autoimmune diseases, allergy and hypersensitivity, measures of altered immune response, and lymphoid cancers. Studies pertaining to effects in humans are presented first, followed by a section discussing relevant studies in animals. The second section of this chapter discusses evidence pertaining to trichloroethylene in relation to lymphoid tissue cancers, including childhood leukemia.

4.6.1. Human Studies

4.6.1.1. *Noncancer Immune-Related Effects*

4.6.1.1.1. ***Immunosuppression, asthma, and allergies.*** In 1982, Lagakos et al. conducted a telephone survey of residents of Woburn, Massachusetts, collecting information on residential history and history of 14 types of medically diagnosed conditions (Lagakos, 1986). The survey included 4,978 children born since 1960 who lived in Woburn before age 19. Completed surveys were obtained from approximately 57% of the town residences with listed phone numbers. Two of the wells providing the town's water supply from 1964 to 1979 had been found to be contaminated with a number of solvents, including tetrachloroethylene (21 ppb) and trichloroethylene (267 ppb) (as cited in [Lagakos, 1986]). Lagakos et al. used information from a study by the Massachusetts Department of Environmental Quality and Engineering to estimate the contribution of water from the two contaminated wells to the residence of each participant, based on zones within the town receiving different mixtures of water from various wells, for the period in which the contaminated wells were operating. This exposure information was used to estimate a cumulative exposure based on each child's length of residence in Woburn. A higher cumulative exposure measure was associated with conditions indicative of immunosuppression (e.g., bacterial or viral infections) or hypersensitivity (e.g., asthma). In contrast, a recent study using the National Health and Nutrition Examination Survey data collected from 1999−2000 in a representative sample of the United States population (n = 550) did not find an association between TCE exposure and self-report of a history of physician-diagnosed asthma (OR: 0.94, 95% CI: 0.77, 1.14) (Arif and Shah, 2007). TCE exposure, as well as exposure to 9 other

volatile organic compounds, was determined through a passive monitor covering a period of 48−72 hours. No clear trend was seen with self-reported wheeze episodes (OR: 1.29, 95% CI: [0.98, 1.68] for one to two episodes; OR: 0.21, 95% CI: [0.04, 10.05] for three or more episodes in the past 12 months).

Allergy and hypersensitivity, as assessed with measures of immune system parameters or immune function tests (e.g., atopy) in humans, have not been extensively studied with respect to the effects of trichloroethylene (see Table 4-58). Lehmann et al. reported data pertaining to IgE levels and response to specific antigens in relation to indoor levels of volatile organic compounds among children (age 36 months) selected from a birth cohort study in Leipzig, Germany (Lehmann et al., 2001). Enrollment into the birth cohort occurred between 1995 and 1996. The children in this allergy study represent a higher-risk group for development of allergic disease, with eligibility criteria that were based on low birth weight (between 1,500 and 2,500 g), or cord blood IgE greater than 0.9 kU/L with double positive family history of atopy. These eligibility criteria were met by 429 children; 200 of these children participated in the allergy study described below, but complete data (IgE and volatile organic compound measurements) were available for only 121 of the study participants. Lehmann et al. measured 26 volatile organic compounds via passive indoor sampling in the child's bedroom for a period of 4 weeks around the age of 36 months. The median exposure of trichloroethylene was 0.42 $\mu g/m^3$ (0.17 $\mu g/m^3$ and 0.87 $\mu g/m^3$ for the 25^{th} and 75^{th} percentiles, respectively). Blood samples were taken at the 36-month-study examination and were used to measure the total IgE and specific IgE antibodies directed to egg white, milk, indoor allergens (house dust mites, cat, molds), and outdoor allergens (timothy-perennial grass, birch- tree). There was no association between trichloroethylene exposure and any of the allergens tested in this study, although some of the other volatile organic compounds (e.g., toluene, 4-ethyltoluene) were associated with elevated total IgE levels and with sensitization to milk or eggs.

4.6.1.1.2. ***Generalized hypersensitivity skin diseases, with or without hepatitis.*** Occupational exposure to trichloroethylene has been associated with a severe, generalized skin disorder that is distinct from contact dermatitis in the clinical presentation of the skin disease (which often involves mucosal lesions), and in the accompanying systemic effects that can include lymphadenopathy, hepatitis, and other organ involvement. Kamijima et al. recently reviewed case reports describing 260 patients with trichloroethylene-related generalized skin disorders (Kamijima et al., 2007). Six of the patients were from the United States or Europe, with the remainder occurring in China, Singapore, Philippines, and other Asian countries. One study in Guangdong province, in southeastern China, included more than 100 of these cases in a single

Table 4-58. Studies of immune parameters (IgE antibodies and cytokines) and trichloroethylene in humans

Parameter, source of data	Results	Reference, location, diagnosis period, sample size, age
IgE antibodies blood sample, indoor air sampling of 28 volatile organic chemicals in child's bedroom	Trichloroethylene exposure not associated with sensitization to indoor or outdoor allergens	Lehmann et al., 2001 Germany 1997−1999. *n* = 121 36-month old children
Cytokine secreting CD3+ T-cell populations cord blood, indoor air sampling of 28 volatile organic chemicals in child's bedroom 4 wks after birth	In CD3+ cord blood cells, some evidence of association between increasing trichloroethylene levels and decreased IL-4 >75th percentile OR: 0.6 (95% CI: 0.2, 2.1), <25th percentile OR 4.4 (95% CI: 1.1, 17.8) increased IFN-γ >75th percentile OR: 3.6 (95% CI: 0.9, 14.9) <25th percentile OR: 0.7 (95% CI: 0.2, 2.2) Similar trends not seen with tumor necrosis factor-α or IL-2	Lehmann et al., 2002 Germany. 1995−1996. *n* = 85 newborns
Cytokine secreting CD3+ and CD8+ T-cell populations blood sample, indoor air sampling of 28 volatile organic chemicals in child's bedroom	Trichloroethylene exposure not associated with percentages of IL-4 CD3+ or IFN-γ CD8+ T-cells	Lehmann et al., 2001 Germany. 1995−1999. *n* = 200 36-month old children.
Cytokine concentration—serum urine sample (trichloroacetic acid concentration), blood sample, questionnaire (smoking history, age, residence), workplace TCE measures (personal samples, 4 exposed and 4 nonexposed workers)	Nonexposed workers similar to office controls for all cytokine measures. Compared to nonexposed workers, the trichloroethylene exposed workers had decreased IL-4 (mean 3.9 vs. 8.1 pg/mL) increased IL-2 (mean 798 vs. 706 pg/mL) increased IFN-γ (mean 37.1 vs. 22.9 pg/mL)	Iavicoli et al., 2005 Italy. *n* = 35 printers using TCE, 30 nonexposed workers (in same factory, did not use or were not near TCE), 40 office worker controls. All men. Mean age ~33 yrs.

year (Huang et al., 2002). Kamijima et al. categorized the case descriptions as indicative of hypersensitivity syndrome (n = 124) or a variation of erythema multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis (n = 115), with 21 other cases unclassified in either category. The fatality rate, approximately 10%, was similar in the two groups, but the prevalence of fever and lymphadenopathy was higher in the hypersensitivity syndrome patients. Hepatitis was seen in 92−94% of the multiforme, Stevens-Johnson syndrome, and toxic epiderma necrolysis patients, but the estimates within the hypersensitivity syndrome group were more variable (46−94%) (Kamijima et al., 2007).

Some of the case reports reviewed by Kamijima et al. provided information on the total number of exposed workers, working conditions, and measures of exposure levels. From the available data, generalized skin disease within a worksite occurred in 0.25 to 13% of workers in the same location, doing the same type of work (Kamijima et al., 2007). The measured concentration of trichloroethylene ranged from <50 mg/m^3 to more than 4,000 mg/m^3, and exposure scenarios included inhalation only and inhalation with dermal exposures. Disease manifestation generally occurred within 2−5 weeks of initial exposure, with some intervals up to 3 months. Most of the reports were published since 1995, and the geographical distribution of cases reflects the newly industrializing areas within Asia.

Kamijima and colleagues recently conducted an analysis of urinary measures of trichloroethylene metabolites (trichloroacetic acid and trichloroethanol) in 25 workers hospitalized for hypersensitivity skin disease in 2002 (Kamijima et al., 2008). Samples taken within 15 days of the last exposure to trichloroethylene exposure were available for 19 of the 25-patients, with a mean time of 8.4 days. Samples from the other patients were not used in the analysis because the half life of urinary trichloroacetic acid is 50−100 hours. In addition, 3−6 healthy workers doing the same type of work in the factories of the affected worker, and 2 control workers in other factories not exposed to trichloroethylene were recruited in 2002−2003 for a study of breathing zone concentration of volatile organochlorines and urinary measures of trichloroethylene metabolites. Worksite measures of trichloroethylene concentration were also obtained. Adjusting for time between exposure and sample collection, mean urinary concentration at the time of last exposure among the 19 patients was 206 mg/mL for trichloroacetic acid. Estimates for trichloroethanol were not presented because of the shorter half-life for this compound. Urinary trichloroacetic acid levels in the healthy exposed workers varied among the 4 factories, with means (±standard deviation [SD]) of 41.6 (±18.0), 131 (±90.2), 180 (±92), and 395 (±684). The lower values were found in a factory in which the degreasing machine had been partitioned from the workers after the illnesses had occurred. Trichloroethylene concentrations (personal time-weighted averages) at the factories of the

affected workers ranged from 164−2,330 mg/m^3 (30−431 ppm). At the two factories with no affected workers in the past 3 years, the mean personal time-weighted average trichloroethylene concentrations were 44.9 mg/m^3 (14 ppm) and 1,803 mg/m^3 (334 ppm). There was no commonality of additives or impurities detected among the affected factories that could explain the occurrence of the hypersensitivity disorder.

To examine genetic influences on disease risk, Dai et al. conducted a case-control study of 111 patients with trichloroethylene-related severe generalized dermatitis and 152 trichloroethylene-exposed workers who did not develop this disease (Dai et al., 2004). Patients were recruited from May 1999 to November 2003 in Guangdong Province, and were employed in approximately 80 electronic and metal-plating manufacturing plants. Initial symptoms occurred within 3 months of exposure. The comparison group was drawn from the same plants as the cases, and had worked for more than 3 months without development of skin or other symptoms. Mean age in both groups was approximately 23 years. A blood sample was obtained from study participants for genotyping of tumor necrosis factor (TNF)-α, TNF-β, and interleukin (IL)-4 genotypes. The genes were selected based on the role of TNF and of interleukin-4 in hypersensitivity and inflammatory responses. The specific analyses included two polymorphisms in the promoter region of TNF-α (G → A substitution at position -308) designated as TNFAII, with wild-type designated TNFAI; and a G → A substitution at position -238), a polymorphism at the first intron on TNF-β, and a polymorphism in the promoter region of IL-4 (C → T substitution at -590). There was no difference in the frequency of the TNF-α^{-238}, TNF-β, or IL-4 polymorphisms between cases and controls, but the wild-type TNF-α^{-308} genotype was somewhat more common among cases (TNF A I/I genotype 94% in cases and 86% in controls).

Kamijima et al. note the similarities, particular with respect to specific skin manifestations, of the case presentations of trichloroethylene-related generalized skin diseases to conditions that have been linked to specific medications (e.g., carbamezepine, allupurinol, antibacterial sulfonamides), possibly in conjunction with reactivation of specific latent herpes viruses (Kamijima et al., 2007). A previous review by these investigators discusses insights with respect to drug metabolism that may be useful in developing hypotheses regarding susceptibility to trichloroethylene-related generalized skin disorders (Nakajima et al., 2003). Based on consideration of metabolic pathways and intermediaries, variability in CYP2E1, UDP-glucoronyltransferase, glutathione-S transferase, and N-acetyl transferase (NAT) activities could be hypothesized to affect the toxicity of trichloroethylene. NAT2 is most highly expressed in liver, and the "slow" acetylation phenotype (which arises from a specific mutation) has been associated with adverse effects of medications, including drug-induced lupus (Lemke and

McQueen, 1995) and hypersensitivity reactions (Spielberg, 1996). There are limited data pertaining to genetic or other sources of variability in these enzymes on risk of trichloroethylene-related generalized skin diseases, however. In a study in Guangdong province, CYP1A1, GSTM1, GSTP1, GSTT1, and NAT2 genotypes in 43 cases of trichloroethylene-related generalized skin disease were compared to 43 healthy trichloroethylene-exposed workers (Huang et al., 2002). The authors reported that the NAT2 slow acetylation genotype was associated with disease, but the data pertaining to this finding were not presented.

4.6.1.1.3. ***Cytokine profiles.*** Cytokines are produced by many of the immune regulatory cells (e.g., macrophages, dendritic cells), and have many different effects on the immune system. The T-helper Type 1 (Th1) cytokines, are characterized as "pro-inflammatory" cytokines, and include TNF-α and interferon (IFN)-γ. Although this is a necessary and important part of the innate immune response to foreign antigens, an aberrant pro-inflammatory response may result in a chronic inflammatory condition and contribute to development of scarring or fibrotic tissue, as well as to autoimmune diseases. Th2 cytokines are important regulators of humoral (antibody-related) immunity. IL-4 stimulates production of IgE and thus influences IgE-mediated effects such as allergy, atopy, and asthma. Th2 cytokines can also act as "brakes" on the inflammatory response, so the balance between different types of cytokine production is also important with respect to risk of conditions resulting from chronic inflammation. Several studies have examined cytokine profiles in relation to occupational or environmental TCE exposure (see Table 4-58).

The 2001 Lehmann et al. study of 36-month old children (described above) also included a blood sample taken at the 3-year study visit, which was used to determine the percentages of specific cytokine producing T-cells in relation to the indoor volatile organic compounds exposures measured at birth. There was no association between trichloroethylene exposure and either IL-4 CD3+ or IFN-γ CD8+ T-cells (Lehmann et al., 2001).

Another study by Lehmann et al. examined the relationship between indoor exposures to volatile organic compounds and T-cell subpopulations measured in cord blood of newborns (Lehmann et al., 2002). The study authors randomly selected 85 newborns (43 boys and 42 girls) from a larger cohort study of 997 healthy, full-term babies, recruited between 1997 and 1999 in Germany. Exclusion criteria included a history in the mother of an autoimmune disease or infectious disease during the pregnancy. Twenty-eight volatile organic compounds were measured via passive indoor sampling in the child's bedroom for a period of 4 weeks after the birth (a period which is likely to reflect the exposures during the prenatal period close to the time of delivery). The levels were generally similar or slightly higher than the levels seen in the previous study using samples from the bedrooms of the 36-month-old children. The highest

levels of exposure were seen for limonene (median 24.3 μg/m^3), α-pinene (median 19.3 μg/m^3) and toluene (median 18.3 μg/m^3), and the median exposure of trichloroethylene was 0.6 μg/m^3 (0.2 μg/m^3 and 1.0 μg/m^3 for the 25th and 75th percentiles, respectively). Flow cytometry was used to measure the presence of CD3 T-cells obtained from the cord blood labeled with antibodies against IFN-γ, tumor necrosis factor-α, IL-2, and IL-4. There was some evidence of a decreased level of IL-2 with higher trichloroethylene exposure in the univariate analysis, with median percentage of IL-2 cells of 46.1 and 33.0% in the groups that were below the 75th percentile and above the 75th percentile of trichloroethylene exposure, respectively. In analyses adjusting for family history of atopy, gender and smoking history of the mother during pregnancy, there was little evidence of an association with either IL-2 or IFN-γ, but there was a trend of increasing trichloroethylene levels associated with decreased IL-4 and increased IFN-γ.

Iavicoli et al. examined cytokine levels in 35 trichloroethylene-exposed workers (Group A) from a printing area of a factory in Italy. Their work involved use of trichloroethylene in degreasing (Iavicoli et al., 2005). Two comparison groups were included. Group B consisted of 30 other factory workers who were not involved in degreasing activities and did not work near this location, and Group C consisted of 40 office workers at the factory. All study participants were male and had worked at their present position for at least 3 years, and all were considered healthy. Personal breathing zone air samples from four workers in Group A and four workers in Group B were obtained in three consecutive shifts (24 total samples) to determine air concentration of trichloroethylene. A urine sample was obtained from each Group A and Group B worker (end of shift at end of work week) for determination of trichloroacetic acid concentrations (corrected for creatinine), and blood samples were collected for assessment of IL-2, IL-4, and IFN-γ concentrations in serum using enzyme-linked immunosorbent assays. Among exposed workers, the mean trichloroethylene concentration was approximately 35 mg/m^3 (30.75 ± SD 9.9, 37.75 ± 23.0, and 36.5 ± 8.2 mg/m^3 in the morning, evening, and night shifts, respectively). The urinary trichloroacetic acid concentrations were much higher in exposed workers compared with nonexposed workers (mean ± SD, Group A 13.3 ± 5.9 mg/g creatinine; Group B 0.02 ± 0.02 mg/g creatinine). There was no difference in cytokine levels between the two control groups, but the exposed workers differed significantly (all *p*-values <0.01 using Dunnett's test for multiple comparisons) from each of the two comparison groups. The observed differences were a decrease in IL-4 levels (mean 3.9, 8.1, and 8.1 pg/mL for groups A, B, and C, respectively), and an increase in IL-2 levels (mean 798, 706, and 730 pg/mL for groups A, B, and C, respectively) and in IFN-γ levels (mean 37.1, 22.9, and 22.8 pg/mL for groups A, B, and C, respectively).

The available data from these studies (Lehmann et al., 2001, 2002; Iavicoli et al., 2005) provide some evidence of an association between increased trichloroethylene exposure and modulation of immune response involving an increase in pro-inflammatory cytokines (IL-2, IFN-γ) and a decrease in Th2 (allergy-related) cytokines (e.g., IL-4). These observations add support to the influence of trichloroethylene in immune-related conditions affected by chronic inflammation.

4.6.1.1.4. *Autoimmune disease*

4.6.1.1.4.1. *Disease clusters and geographic-based studies*. Reported clusters of diseases have stimulated interest in environmental influences on systemic autoimmune diseases. These descriptions include investigations into reported clusters of systemic lupus erythematosus (Balluz et al., 2001; Dahlgren et al., 2007) and Wegener granulomatosis (Albert et al., 2005). Wegener granulomatosis, an autoimmune disease involving small vessel vasculitis, usually with lung or kidney involvement, is a very rare condition, with an incidence rate of 3−14 per million per year (Mahr et al., 2006). Trichloroethylene was one of several ground water contaminants identified in a recent study investigating a cluster of seven cases of Wegener granulomatosis around Dublin, Pennsylvania. Because of the multiple contaminants, it is difficult to attribute the apparent disease cluster to any one exposure.

In addition to the study of asthma and infectious disease history among residents of Woburn, Massachusetts (Lagakos, 1986) (see Section 4.6.1.1.1), Byers et al. provide data pertaining to immune function from 23 family members of leukemia patients in Woburn, Massachusetts (Byers et al., 1988). Serum samples were collected in May and June of 1984 and in November of 1985 (several years after 1979, when the contaminated wells had been closed). Total lymphocyte counts and lymphocyte subpopulations (CD3, CD4, and CD8) and the CD4/CD8 ratio were determined in these samples, and in samples from a combined control group of 30 laboratory workers and 40 residents of Boston selected through a randomized probability area sampling process. The study authors also assessed the presence of antinuclear antibodies (ANA) or other autoantibodies (antismooth muscle, antiovarian, antithyroglobulin, and antimicrosomal antibodies) in the family member samples and compared the results with laboratory reference values. The age distribution of the control group, and stratified analyses by age, are not provided. The lymphocyte subpopulations were higher and the CD4/CD8 ratio was lower in the Woburn family members compared to the controls in both of the samples taken in 1984. In the 1985 samples, however, the subpopulation levels had decreased and the CD4/CD8 ratio had increased; the values were no longer statistically different from the controls. None of the family member serum samples had antithyroglobulin or antimicrosomal antibodies, but

10 family-member serum samples (43%) had ANA (compared to <5% expected based on the reference value). Because the initial blood sample was taken in 1984, it is not possible to determine the patterns at a time nearer to the time of the exposure. The coexposures that occurred also make it difficult to infer the exact role of trichloroethylene in any alterations of the immunologic parameters.

Kilburn and Warshaw reported data from a study of contamination by metal-cleaning solvents (primarily trichloroethylene) and heavy metals (e.g., chromium) of the aquifer of the Santa Cruz River in Tucson, Arizona (Kilburn and Warshaw, 1992). Exposure concentrations above 5 ppb (6−500 ppb) had been documented in some of the wells in this area. A study of neurological effects was undertaken between 1986 and 1989 (Kilburn and Warshaw, 1993), and two of the groups within this larger study were also included in a study of symptoms relating to systemic lupus erythematosus. Residents of Tucson (n = 362) were compared to residents of southwest Arizona (n = 158) recruited through a Catholic parish. The Tucson residents were selected from the neighborhoods with documented water contamination (>5 ppb trichloroethylene for at least one year between 1957 and 1981). Details of the recruitment strategy are not clearly described, but the process included recruitment of patients with lupus or other rheumatic diseases (Kilburn and Warsaw, 1993, 1992). The prevalence of some self-reported symptoms (malar rash, arthritis/arthralgias, Raynaud syndrome, skin lesions, and seizure or convulsion was significantly higher in Tucson, but there was little difference between the groups in the prevalence of oral ulcers, anemia, low white blood count or low platelet count, pleurisy, alopecia, or proteinuria. The total number of symptoms reported was higher in Tucson than in the other southwest Arizona residents (14.3 vs. 6.4% reported four or more symptoms, respectively). Low-titer (1:80) ANA were seen in 10.6 and 4.7% of the Tucson and other Arizona residents, respectively (p = 0.013). However, since part of the Tucson study group was specifically recruited based on the presence of rheumatic diseases, it is difficult to interpret these results.

4.6.1.1.4.2. ***Case-control studies*****.** Interest in the role of organic solvents, including trichloroethylene, in autoimmune diseases was spurred by the observation of a scleroderma-like disease characterized by skin thickening, Raynaud's phenomenon, and acroosteolysis and pulmonary involvement in workers exposed to vinyl chloride (Gama and Meira, 1978). A case report in 1987 described the occurrence of a severe and rapidly progressive case of systemic sclerosis in a 47-year-old woman who had cleaned X-ray tubes in a tank of trichloroethylene for approximately 2.5 hours (Lockey et al., 1987).

One of the major impediments to autoimmune disease research is the lack of disease registries, which make it difficult to identify incident cases of specific diseases (NIAMS, 2007). There are no cohort studies of the incidence of autoimmune diseases in workers exposed to trichloroethylene. Most of the epidemiologic studies of solvents and autoimmune disease rely on general measures of occupational exposures to solvents, organic solvents, or chlorinated solvents exposures. A 2- to 3-fold increased risk of systemic sclerosis (scleroderma) (Aryal et al., 2001; Garabrant et al., 2003; Maitre et al., 2004), rheumatoid arthritis (Lundberg et al., 1994; Sverdrup et al., 2005), undifferentiated connective tissue disease (Lacey et al., 1999), and antineutrophil-cytoplasmic antibody (ANCA)-related vasculitis (Beaudreuil et al., 2005; Lane et al., 2003) has generally been seen in these studies, but there was little evidence of an association between solvent exposure and systemic lupus erythematosus in two recent case-control studies (Cooper et al., 2004; Finckh et al., 2006).

Two case-control studies of scleroderma (Bovenzi et al., 2004; Maitre et al., 2004) and two of rheumatoid arthritis (Olsson et al., 2004, 2000) provide data concerning solvent exposure that occurred among metal workers or in jobs that involved cleaning metal (i.e., types of jobs which were likely to use trichloroethylene as a solvent). There was a 2-fold increased risk among male workers in the two studies of rheumatoid arthritis from Sweden (Olsson et al., 2004, 2000). The results from the smaller studies of scleroderma were more variable, with no exposed cases seen in one study with 93 cases and 206 controls (Maitre et al., 2004), and an odds ratio of 5.2 (95% CI: 0.7, 37) seen in a study with 56 cases and 171 controls (Bovenzi et al., 2004).

Five other case-control studies provide data specifically about trichloroethylene exposure, based on industrial hygienist review of job history data (see Table 4-59). Three of these studies are of scleroderma (Diot et al., 2002; Garabrant et al., 2003; Nietert et al., 1998), one is of undifferentiated connective tissue disease (Lacey et al., 1999), and one is of small vessel vasculitidies involving ANCAs (Beaudreuil et al., 2005).

These studies included some kind of expert review of job histories, but only two studies included a quantification of exposure (e.g., a cumulative exposure metric, or a “high” exposure group) (Diot et al., 2002; Nietert et al., 1998). Most of the studies present data stratified by sex, and as expected, the prevalence of exposure (either based on type of job or on industrial hygienist assessment) is considerably lower in women compared with men. In men the studies generally reported odds ratios between 2.0 and 8.0, and in women, the odds ratios were between 1.0 and 2.0. The incidence rate of scleroderma in the general population is approximately 5−10 times higher in women compared with men, which may make it easier to detect large relative risks in men.

Table 4-59. Case-control studies of autoimmune diseases with measures of trichloroethylene exposure

Disease, source of data	Results: exposure prevalence, OR, 95% CI	Reference, location, sample size, age
Scleroderma		
Structured interview (specific jobs and materials; jobs held 1 or more years). Exposure classified by self-report and by expert review (job exposure matrix).	Men Maximum intensity 30% cases, 10% controls OR: 3.3 (1.0, 10.3) Cumulative intensity 32% cases, 21% controls OR: 2.0 (0.7, 5.3) Maximum probability 16% cases, 3% controls OR: 5.1 (not calculated) Women: Maximum intensity 6% cases, 7% controls OR: 0.9 (0.3, 2.3) Cumulative intensity 10% cases, 9% controls OR: 1.2 (0.5, 2.6) Maximum probability 4% cases, 5% controls OR: 0.7 (0.2, 2.2)	Nietert et al., 1998 South Carolina. Prevalent cases, 178 cases (141 women, 37 men), 200 hospital-based controls. Mean age at onset 45.2 yrs.
Structured interview (specific jobs and materials; jobs held 6 or more months). Exposure classified by expert review.	Men and women any exposure: cases 16%, controls 8% OR: 2.4 (95% CI: 1.0, 5.4) high exposure:[a] cases 9%, controls 1% OR: 7.6 (95% CI: 1.5, 37.4) Men any exposure: cases 64%, controls 27% OR: 4.7 (95% CI: 0.99, 22.0) Women any exposure: cases 9%, controls 4% OR: 2.1 (95% CI: 0.65, 6.8)	Diot et al., 2002 France. Prevalent cases, 80 cases (69 women, 11 men), 160 hospital controls. Mean age at diagnosis 48 yrs.
Structured interview (specific jobs and materials; jobs held 3 or more months). Exposure classified by self-report and by expert review.	Women Self report: cases 1.3%, controls 0.7% OR: 2.0 (95% CI: 0.8, 4.8) Expert review: cases 0.7%, controls 0.4% OR: 1.9 (95% CI: 0.6, 6.6)	Garabrant et al., 2003 Michigan and Ohio. Prevalent cases, 660 cases (all women), 2,227 population controls.[b] Ages 18 and older.
Undifferentiated connective tissue disease		
Structured interview (specific jobs and materials; jobs held 3 or more months). Exposure classified by self-report and by expert review.	Women Self report: cases 0.5%, controls 0.7% OR: 0.88 (95% CI: 0.11, 6.95) Expert review: cases 0.5%, controls 0.4% OR: 1.67 (95% CI: 0.19, 14.9)	Lacey et al., 1999 Michigan and Ohio. Prevalent cases, 205 cases (all women), 2,095 population controls. Ages 18 and older.

Table 4-59. Case-control studies of autoimmune diseases with measures of trichloroethylene exposure (continued)

Disease, source of data	Results: exposure prevalence, OR, 95% CI	Reference, location, sample size, age
ANCA-related diseases[c]		
Structured interview (specific jobs and materials; jobs held 6 or more months). Exposure classified by expert review.	Men and women (data not presented separately by sex) cases 18.3%, controls 17.5% OR: 1.1 (0.5, 2.4)	Beaudreuil et al., 2005 France. Incident cases, 60 cases (~50% women), 120 hospital controls. Mean age 61 yrs.

[a]Cumulative exposure defined as product of probability × intensity × frequency × duration scores, summed across all jobs; scores of >1 classified as "high."
[b]Total *n*; *n* with TCE data: self -report 606 cases, 2,138 control; expert review 606 cases, 2,137 controls.
[c]Diseases included Wegener glomerulonephritis (*n* = 20), microscopic polyangiitis (*n* = 8), pauci-immune glomerulonephritis (*n* = 10), uveitis (*n* = 6), Churg-Strauss syndrome (*n* = 4), stroke (*n* = 4) and other diseases (no more than 2 each).

The U.S. EPA conducted a meta-analysis of the three scleroderma studies with specific measures of trichloroethylene (Diot et al., 2002; Garabrant et al., 2003; Nietert et al., 1998), examining separate estimates for males and for females. The resulting combined estimate for "any" exposure, using a random effects model to include the possibility of nonrandom error between studies (DerSimonian and Laird, 1986), was OR: 2.5 (95% CI: 1.1, 5.4) for men and OR: 1.2 (95% CI: 0.58, 2.6) in women. (Because the "any" exposure variable was not included in the published report, Dr. Paul Nietert provided the U.S. EPA with a new analysis with these results, e-mail communication from Paul Nietert to Glinda Cooper, November 28, 2007.)

Specific genes may influence the risk of developing autoimmune diseases, and genes involving immune response (e.g., cytokines, major histocompatibility complex, B- and T-cell activation) have been the focus of research pertaining to the etiology of specific diseases. The metabolism of specific chemical exposures may also be involved (Cooper et al., 1999). Povey et al. (2001) examined polymorphisms of two cytochrome CYP genes, CYP2E1 and CYP2C19, in relation to solvent exposure and risk of developing scleroderma. These specific genes were examined because of their hypothesized role in metabolism of many solvents, including trichloroethylene. Seven scleroderma patients who reported a history of solvent exposure were compared to 71 scleroderma patients with no history of solvent exposure and to 106 population-based controls. The CYP2E1*3 allele and the CYP2E1*4 allele were more common in the 7 solvent-exposed patients (each seen in 2 of the 7 patients; 29%) than in either of the comparison groups (approximately 5% for CYP2E1*3 and 14% for CYP2E1*4). The authors present these results as observations that require a larger study for corroboration and further elucidation of specific interactions.

4.6.1.2. *Cancers of the Immune System, Including Childhood Leukemia*

4.6.1.2.1. ***Description of studies.*** Human studies have reported cancers of the immune system resulting from TCE exposure. Lymphoid tissue neoplasms arise in the immune system and result from events that occur within immature lymphoid cells in the bone marrow or peripheral blood (leukemias), or more mature cells in the peripheral organs (non-Hodgkin's lymphoma, NHL). As such, the distinction between lymphoid leukemia and NHL is largely distributional with overlapping entities, such that a particular lymphoid neoplasm may manifest both lymphomatous and leukemic features during the course of the disease (Weisenberger, 1992). Lymphomas are grouped according to the World Health Organization (WHO) classification as B-cell neoplasms, T-cell/ natural killer (NK)-cell neoplasms, and Hodgkin's lymphoma, formerly known as Hodgkin's disease (Harris et al., 2000).

Numerous studies are found in the published literature on lymphoma and either broad exposure categories or occupational title. Most of these studies evaluate NHL, specifically. The NHL studies generally report positive associations with organic solvents or job title as aircraft mechanic, metal cleaner or machine tool operator, and printers, although associations are not observed consistently across all studies, specific solvents are not identified, and different lymphoma classifications are adopted (Alexander et al., 2007; Blair et al., 1993; Boffetta and de Vocht, 2007; Chiu and Weisenburger, 2003; Dryver et al., 2004; Figgs et al., 1995; Karunanayake et al., 2008; Lynge et al., 1997; Richardson et al., 2008; Seidler et al., 2007; Mannetje et al., 2008; Tatham et al., 1997; Vineis et al., 2007; Schenk et al., 2009; Wang et al., 2009). A major use of TCE is the degreasing as vapor or cold state solvent of metal and other products with potential exposure in jobs in the metal industry, printing industry and aircraft maintenance or manufacturing industry (Bakke et al., 2007). The recent NHL case-control study of Purdue et al. (2009) examined degreasing tasks, specifically, and reported an increasing positive trend between NHL risk in males and three degreasing exposure surrogates: average frequency (hours/year) ($p = 0.02$), maximal frequency (hours/year), ($p = 0.06$), or cumulative number of hours($p = 0.04$).

As described in Appendix B, the U.S. EPA conducted a thorough and systematic search of published epidemiological studies of cancer risk and trichloroethylene exposure using the PubMed, ToxNet, and EMBASE bibliographic database. The U.S. EPA also requested unpublished data pertaining to trichloroethylene from studies that may have collected these data but did not include it in their published reports. ATSDR and state health department peer-reviewed studies were also reviewed. Information from each of these studies relating to specified design and analysis criteria was abstracted. These criteria included aspects of study design, representativeness of study subjects, participation rate/loss to follow-up, latency considerations, potential for biases related to exposure misclassification, disease misclassification, and surrogate information, consideration of possible confounding, and approach to statistical analysis. All studies are considered for hazard identification but those studies more fully meeting the objective criteria provided the greater weight for identifying a cancer hazard.

The body of evidence on lymphoma and trichloroethylene is comprised of occupational cohort studies, population-based case-control studies and geographic studies. Four case-control studies and four geographic studies also examine childhood leukemia and trichloroethylene. Most studies report observed risk estimates and associated confidence intervals for lymphoma and overall TCE exposure. The 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. (1999) 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., 2005) 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). Fewer studies presented in published papers this information for cell-specific lymphomas, leukemia, leukemia cell type, or multiple myeloma.

The seven cohort studies with data on the incidence of lymphopoietic and hematopoietic cancer in relation to trichloroethylene exposure range in size (803 [Hansen et al., 2001] to 86,868 [Chang et al., 2005]), and were conducted in Denmark, Sweden, Finland, Taiwan and the United States (see Table 4-60; for additional study descriptions, see Appendix B). 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-Nielsen et al., 2003). The exposure assessment techniques used in all studies except Chang et al. (2005) and Sung et al. (2007) included a detailed job exposure matrix (Zhao et al., 2005; Blair et al., 1998), biomonitoring data (Anttila et al., 1995; Axelson et al., 1994; Hansen et al., 2001), or reference to industrial hygiene records on TCE exposure patterns and factors that affected exposure, indicating a high probability of TCE exposure potential (Raaschou-Nielsen et al, 2003) with high probability of TCE exposure to individual subjects. Subjects in Chang et al. (2005) and Sung et al. (2007), two studies with overlapping subjects employed at an electronics plant in Taiwan, have potential exposure to several solvents including TCE; all subjects are presumed as "exposed" because of employment in the plant although individual subjects would be expected to have differing exposure potentials. The lack of attribution of exposure intensity to individual subjects yields a

greater likelihood for exposure misclassification compared to the six other studies with exposure assessment approaches supported by information on job titles, tasks, and industrial hygiene monitoring data. 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, observations must be interpreted in light of possible bias reflecting incomplete ascertainment of incident cases.

Eighteen cohort or PMR studies describing mortality risks from lymphopoietic and hematopoietic cancer are summarized in Table 4-61 (for additional study descriptions, see Appendix B)**.** Two studies examined cancer incidence and are identified above (Blair et al., 1998; Zhao et al., 2005). In 10 of the 18 studies presenting mortality risks (Blair et al., 1989; Chang et al., 2003; Costa et al., 1989; Garabrant et al., 1988; Henschler et al., 1995; Sinks et al., 1992; Sung et al., 2007; Wilcosky et al., 1984; ATSDR, 2004; Clapp and Hoffman, 2008), a relatively limited exposure assessment methodology was used, study participants may not represent the underlying population, or there was a low exposure prevalence of TCE exposure. For reasons identified in the systematic review, these studies are given less weight in the overall evaluation of the literature than the eight other cohort studies that better met the ideals of evaluation criteria (Blair et al., 1998 and extended follow-up by Radican et al., 2008; Boice et al., 1999, 2006; Greenland et al., 1994; Morgan et al., 1998; Ritz, 1999; Zhao et al., 2005).

Table 4-60. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk

Population exposure group		Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
		Relative risk (95% CI)[a]	*n*[a]	Relative risk (95% CI)[a]	*n*[a]	Relative risk (95% CI)[a]	*n*[a]	
Aerospace workers (Rocketdyne), CA								Zhao et al., 2005
	Any TCE exposure	Not reported		Not reported				*n* = 5,049 (2,689 with high cumulative TCE exposure), began work before 1980, worked at least 2 yrs, alive with no cancer diagnosis in 1988, follow-up from 1988−2000, job exposure matrix (intensity), internal referents (workers with no TCE exposure). Leukemia observations included in non-Hodgkin lymphoma category
	Low cumulative TCE score			1.0 (referent)	28			
	Medium cumulative TCE score			0.88 (0.47, 1.65)	16			
	High cumulative TCE score			0.20 (0.03, 1.46)	1			
	(*p* for trend)			(0.097)				
Electronic workers, Taiwan								Chang et al., 2005; Sung et al., 2007
	All employees	0.67 (0.42, 1.01)	22					*n* = 88,868 (*n* = 70,735 female), follow-up 1979−1997, does not identify TCE exposure to individual subjects (Chang et al., 2005)
	Males	0.73 (0.27, 1.60)	6	Not reported		Not reported		
	Females	0.65 (0.37, 1.05)	16	Not reported		Not reported		
	Females					0.78 (0.49, 1.17)	23	*n* = 63,982 females, follow-up 1979−2001, dose not identify TCE exposure to individual subjects (Sung et al., 2007)
Blue-collar workers, Denmark								Raaschou-Nielsen et al., 2003
	Any exposure	1.1 (1.0, 1.6)	229	1.2 (1.0, 1.5)	96	1.2 (0.9, 1.4)	82	*n* = 40,049 (14,360 with presumed higher level exposure to TCE), worked for at least 3 months, follow-up from 1968−1997, documented TCE use[c]. U.S. EPA based the lymphopoietic cancer category on summation of ICD codes 200−204.
	Subcohort w/higher exposure[d]	Not reported		1.5 (1.2, 2.0)	65	Not reported		
	Employment duration							
	1−4.9 yrs			1.5 (1.1, 2.1)	35			
	≥5 yrs			1.6 (1.1, 2.2)	30			

Table 4-60. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population exposure group		Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
		Relative risk (95% CI)[a]	*n*[a]	Relative risk (95% CI)[a]	*n*[a]	Relative risk (95% CI)[a]	*n*[a]	
Biologically-monitored workers, Denmark								Hansen et al., 2001
	Any TCE exposure	2.0 (1.1, 3.3)	15	3.1 (1.3, 6.1)	8	2.0 (0.7, 4.4)	6	*n* = 803, U-TCA or air TCE samples, follow-up 1968−1996 (subset of Raaschlou-Nielsen et al. [2003] cohort). U.S. EPA based the lymphopoietic cancer category on summation of ICD codes 200−204
	Cumulative exposure (Ikeda), males	Not reported				Not reported		
	<17 ppm-yr			3.9 (0.8, 11)	3			
	≥17 ppm-yr			3.1 (0.6, 9.1)	3			
	Mean concentration (Ikeda), males	Not reported				Not reported		
	<4 ppm			3.9 (1.1, 10)	4			
	4+ ppm			3.2 (1.1, 10)	4			
	Employment duration, males	Not reported				Not reported		
	<6.25 yr			2.5 (0.3, 9.2)	2			
	≥6.25 yr			4.2 (1.1, 11)	4			
Aircraft maintenance workers, Hill Air Force Base, UT								Blair et al., 1998
	TCE Subcohort	Not reported		Not reported		Not reported		*n* = 10,461 men and 3,605 women (total *n* = 14,066, *n* = 7,204 with TCE exposure), employed at least 1 yr from 1952 to 1956, follow-up 1973−1990, job exposure matrix (intensity), internal referent (workers with no chemical exposures)
	Males, cumulative exposure		36		19		7	
	0	1.0 (referent)		1.0 (referent)		1.0 (referent)		
	<5 ppm-yr	0.8 (0.4, 1.7)	12	0.9 (0.3, 2.6)	8	0.4 (0.1, 2.0)	2	
	5−25 ppm-yr	0.7 (0.3, 1.8)	7	0.7 (0.2, 2.6)	4		0	
	>25 ppm-yr	1.4 (0.6, 2.9)	17	1.0 (0.4, 2.9)	7	0.9 (0.2, 3.7)	4	
	Females, cumulative exposure							
	0	1.0 (referent)		1.0 (referent)		1.0 (referent)		
	<5 ppm-yr	1.2 (0.3, 4.4)	3	0.6 (0.1, 5.0)	1		0	
	5−25 ppm-yr	1.9 (0.4, 8.8)	2		0	2.4 (0.3, 21.8)	1	
	>25 ppm-yr	0.9 (9.2, 3.3)	3	0.9 (0.2, 4.5)	2		0	

Table 4-60. Incidence cohort studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population exposure group		Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
		Relative risk (95% CI)[a]	*n*[a]	Relative risk (95% CI)[a]	*n*[a]	Relative risk (95% CI)[a]	*n*[a]	
Biologically-monitored workers, Finland		1.51 (0.92, 2.33)	20	1.81 (0.78, 3.56)	8	1.08 (0.35, 2.53)	5	Anttila et al., 1995
	Mean air-TCE (Ikeda extrapolation)							*n* = 3,089 men and women, U-TCA samples, follow-up 1967−1992
	<6 ppm	1.36 (0.65, 2.49)	10	2.01 (0.65, 4.69)	5	0.39 (0.01, 2.19)	1	
	6+ ppm	2.08 (0.95, 3.95)	9	1.40 (0.17, 5.04)	2	2.65 (0.72, 6.78)	4	
Biologically-monitored workers, Sweden								Axelson et al., 1994
	Males, 2+ yrs exposure duration	1.17 (0.47, 2.40)	7	1.56 (0.51, 3.64)	5	Not reported		*n* = 1,421 men and 249 women (total 1,670), U-TCA samples, follow-up 1958−1987. U.S. EPA based the lymphopoietic cancer category includes ICD-7 200−203.
	0−17 ppm (Ikeda extrapolation)	Not reported		1.44 (0.30, 4.20)	3	Not reported		
	18−35 ppm (Ikeda extrapolation)			(0, 8.58)	0			
	≥36 ppm (Ikeda extrapolation)			6.25 (0.16, 34.8)	1			
	Females	Not reported		Not reported		Not reported		

[a] *n* = number of observed cases.
[b]Standardized incidence ratios using an external population referent group unless otherwise noted.
[c] Exposure assessment based on industrial hygiene data on TCE exposure patterns and factors that affect such exposure (Raaschou-Nielsen et al., (2002), with high probability of TCE exposure potential to individual subjects. Companies included iron and metal (48%), electronics (11%), painting (11%), printing (8%), chemical (5%), dry cleaning (5%), and other industries.
[d]Defined as at least 1 year duration and first employed before 1980.

Table 4-61. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk

Population, exposure group	Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	
Computer manufacturing workers (IBM), NY							Clapp and Hoffman, 2008
Males	2.24 (1.01, 4.19)	9					*n* = 115 cancer deaths from 1969−2001, proportional cancer mortality ratio, does not identify TCE exposure to individual subjects. U.S. EPA based the lymphopoietic cancer category on "all lymphatic cancers."
Females		0					
Aerospace workers (Rocketdyne), CA							
Any TCE (utility/eng flush)	0.74 (0.34, 1.40)	9	0.21 (0.01, 1.18)	1	1.08 (0.35, 2.53)	5	Boice et al., 2006
							n = 41,351 (1,111 Santa Susana workers with any TCE exposure), employed on or after 1948−1999, worked ≥6 months, follow-up to 1999, job exposure matrix without quantitative estimate of TCE intensity.
Any TCE exposure	Not reported		Not reported	60	Not reported		Zhao et al., 2005
Low cumulative TCE score	Not reported		1.0 (referent)	27			*n* = 6,044 (*n* = 2,689 with high cumulative level exposure to TCE), began work and worked at least 2 yrs in 1950 or later - 1993, follow-up to 2001, job exposure matrix (intensity), internal referents (workers with no TCE exposure). Leukemia observations included in non-Hodgkin lymphoma category.
Medium cumulative TCE score			1.49 (0.86, 2.57)	27			
High TCE score			1.30 (0.52, 3.23)	6			
(*p* for trend)			(0.370)				

Table 4-61. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	
View-Master employees, OR							ATSDR, 2004
Males	0.58 (0.11, 1.69)	3	0.69 (0.08, 2.49)	2	0.50 (0.01, 2.79)	1	*n* = 616 deaths from 1989−2001, proportional mortality ratio, does not identify TCE exposure to individual subjects. U.S. EPA based the non-Hodgkin lymphoma cancer category on "other lymphopoietic tissue."
Females	0.64 (0.28, 1.26)	8	0.52 (0.14, 1.33)	4	0.67 (0.14, 1.96)	3	
Electronic workers, Taiwan							Chang et al., 2003
All employees							*n* = 88,868 (*n* = 70,735 female), began work 1978−1997, follow-up 1985−1997, does not identify TCE exposure to individual subjects.
Males	Not reported		1.27 (0.41, 2.97)	5	0.44 (0.05, 1.59)	2	
Females	Not reported		1.14 (0.55, 2.10)	10	0.54 (0.23, 1.07)	8	
Aerospace workers (Lockheed), CA							
Routine TCE, any exposure	1.5 (0.81, 1.60)	36	1.19 (0.65, 1.99)	14	1.05 (0.54, 1.84)	12	Boice et al., 1999
Routine-intermittent							*n* = 77,965 (*n* = 2,267 with routine TCE exposure and *n* = 3.016 with intermittent-routine TCE exposure), began work ≥1960, worked at least 1 yr, follow-up from 1960−1996, job exposure matrix without quantitative estimate of TCE intensity.
Any TCE exposure	Not reported		Not reported		Not reported		
Duration of exposure	Not reported				Not reported		
0 yrs			1.0 (referent)	32			
<1 yr			0.74 (0.32, 1.72)	7			
1−4 yrs			1.33 (0.64, 2.78)	10			
≥5 yrs			1.62 (0.82, 3.22)	14			
p for trend			0.20				

Table 4-61. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group		Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
		Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	
Uranium-processing workers (Fernald), OH								Ritz, 1999
	Any TCE exposure	Not reported		Not reported		Not reported		*n* = 3,814 (*n* = 2,971 with TCE), began work 1951−1972, worked ≥3 months, follow-up to 1989, internal referents (workers with no TCE exposure).
	No TCE exposure	1.0 (referent)		Not reported		Not reported		
	Light TCE exposure, >2 yrs	1.45 (0.68, 3.06)[c]	18	Not reported		Not reported		
	Moderate TCE exposure, >2 yrs	1.17 (0.15, 9.00)[c]	1	Not reported		Not reported		
Aerospace workers (Hughes), CA								Morgan et al., 1998
.	TCE subcohort	0.99 (0.64, 1.47)	25	0.96 (0.20, 2.81)[d]	3	1.05 (0.50, 1.93)	10	*n* = 20,508 (4,733 with TCE exposure), worked ≥6 months 1950−1985, follow-up to 1993, external and internal (all non-TCE exposed workers) workers referent, job exposure matrix (intensity).
	TCE subcohort			1.01 (0.46, 1.92)[e]	9			
	Low intensity (<50 ppm)	1.07 (0.51, 1.96)	10	1.79 (0.22, 6.46)[d]	2	0.85 (0.17, 2.47)	3	
	High intensity (>50 ppm)	0.95 (0.53, 1.57)	15	0.50 (0.01, 2.79)[d]	1	1.17 (0.47, 2.41)	7	
	TCE subcohort (Cox Analysis)							
	Never exposed	1.0 (referent)	82	1.0 (referent)	8	1.0 (referent)	32	
	Ever exposed	1.05 (0.67, 1.65)[f]	25	1.36 (0.35, 5.22)[d, f]	3	0.99 (0.48, 2.03)[f]	10	
	Peak							
	No/Low	1.0 (referent)	90	1.0 (referent)	9	1.0 (referent)	35	
	Medium/High	1.08 (0.64, 1.82)	17	1.31 (0.28, 6.08)[d]	2	1.10 (0.49, 2.49)	7	
	Cumulative							
	Referent	1.0 (referent)	82	1.0 (referent)	8	1.0 (referent)	32	
	Low	1.09 (0.56, 2.14)	10	2.25 (0.46, 11.1)[d]	2	0.69 (0.21, 2.32)	3	
	High	1.03 (0.59, 1.79)	15	0.81 (0.10, 6.49)[d]	1	1.14 (0.5, 2.60)	7	

Table 4-61. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	
Aircraft maintenance workers, Hill Air Force Base, UT							Blair et al., 1998; Radican et al., 2008
TCE subcohort	1.1 (0.7, 1.8)[g]	66	2.0 (0.9, 4.6)[g]	28	0.6 (0.3, 1.2)[g]	16	*n* = 14,066 (*n* = 7,204 ever exposed to TCE), employed at least 1 yr from 1952 to 1956, follow-up to 1990 (Blair et al., 1998) or to 2000 (Radican et al., 2008), job exposure matrix, internal referent (workers with no chemical exposures).
Males, cumulative exposure							
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.1 (0.6, 2.1)	21	1.8 (0.6, 5.4)	10	1.0 (0.3, 3.2)	7	
5−25 ppm-yr	1.0 (0.4, 2.1)	11	1.9 (0.6, 6.3)	6		0	
>25 ppm-yr	1.3 (0.7, 2.5)	21	1.1 (0.3, 3.8)	5	1.2 (0.4, 3.6)	7	
Females, cumulative exposure							
0	1.0 (referent)				1.0 (referent)		
<5 ppm-yr	1.5 (0.6, 4.0)	6	3.8 (0.8, 18.9)	3	0.4 (0.1, 3.2)	1	
5−25 ppm-yr	0.7 (0.1, 4.9)	1		0		0	
>25 ppm-yr	1.1 (0.4, 3.0)	6	3.6 (0.8, 16.2)	4	0.3 (0.1, 2.4)	1	
TCE subcohort	1.06 (0.75, 1.51)[h]	106	1.36 (0.77, 2.39)[h]	46	0.64 (0.35, 1.18)[h]	27	
Males, cumulative exposure	1.12 (0.72, 1.73)	88	1.56 (0.79, 4.21)	37	0.77 (0.37, 1.62)	24	
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.04 (0.63, 1.74)	34	1.83 (0.79, 4.21)	18	0.86 (0.36, 2.02)	11	
5−25 ppm-yr	1.06 (0.49, 1.88)	21	1.17 (0.42, 3.24)	7	0.51 (0.16, 1.63)	4	
>25 ppm-yr	1.25 (0.75, 2.09)	33	1.50 (0.61, 3.69)	12	0.87 (0.35, 2.14)	9	
Females, cumulative exposure	1.00 (0.55, 1.83)	18	1.18 (0.49, 2.85)	9	0.36 (0.10, 1.32)	3	
0	1.0 (referent)		1.0 (referent)		1.0 (referent)		
<5 ppm-yr	1.10 (0.48, 2.54)	7	1.48 (0.47, 4.66)	4	0.35 (0.05, 2.72)	1	
5−25 ppm-yr	0.38 (0.05, 2.79)	1		0		0	
>25 ppm-yr	1.11 (0.53, 2.31)	10	1.30 (0.45, 3.77)	5	0.48 (0.10, 2.19)	2	

Table 4-61. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	
Cardboard manufacturing workers, Arnsburg, Germany							Henschler et al., 1995
TCE-exposed subjects	1.10 (0.03, 6.12)	1					*n* = 169 TCE exposed and *n* = 190 unexposed men, employed ≥1 yr from 1956−1975, follow-up to 1992, local population referent, qualitative exposure assessment.
Unexposed subjects from same factory	1.11 (0.03, 6.19)	1					
General Electric plant, Pittsfield, MA			0.76 (0.24, 2.42)[i, j]	15	1.1 (0.46, 2.66)[i]	22	Greenland et al., 1994
							Nested case-control study, *n* = 512 cancer (cases) and 1,202 noncancer (controls) male deaths reported to pension fund between 1969−1984 among workers employed <1984 and with job history record, job exposure matrix-ever held job with TCE exposure.
Cardboard manufacturing workers, Atlanta, GA							Sinks et al., 1999
	0.3 (0.0, 1.6)	1	Not reported		Not reported		*n* = 2,050, employed on or before 1957−1988, follow-up to 1988, Material Data Safety Sheets used to identify chemicals used in work areas.
U. S, Coast Guard employees							Blair et al., 1988
Marine inspectors	1.57 (0.91, 2.51)	17	1.75 (0.48, 4.49)	4	1.55 (0.62, 3.19)	7	n=3,781 males (1,767 marine inspectors), employed 1942-1970, follow-up to 1980. TCE and nine other chemicals identified as potential exposures; no exposure assessment to individual subjects.
Noninspectors	0.60 (0.24, 1.26)	7	0.41 (0.01, 2.30)	1	0.66 (0.14, 1.94)	3	

Table 4-61. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	
Aircraft manufacturing employees, Italy							Costa et al., 1989
All male subjects	0.80 (0.41, 1.40)	12	Not reported		Not reported		*n* = 7,676 males, employed on or before 1954−1981, followed to 1981, job titles of white- and blue-collar workers, technical staff, and admin. clerks, does not identify TCE exposure to individual subjects.
Aircraft manufacturing, San Diego, CA							Garabrant et al., 1988
All employees	0.82 (0.56, 1.15)	32	0.82 (0.44, 1.41)[d]	13	0.82 (0.47, 1.32)	10	*n* = 14,067, employed at least 4 yrs with company and ≥1 d at San Diego plant from 1958−1982, followed to 1982, does not identify TCE exposure to individual subjects.
			0.65 (0.21, 1.52)[k]	5			

Table 4-61. Mortality cohort and PMR studies of TCE exposure and lymphopoietic and hematopoietic cancer risk (continued)

Population, exposure group	Lymphopoietic cancer		non-Hodgkin lymphoma		Leukemia		Reference(s) and study description[b]
	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	Relative risk (95% CI)	*n*[a]	
Solvent-exposed rubber workers	2.4[i]	3	0.81	3			Wilcosky et al., 1984
							Nested case-control study, *n* = 9 lymphosarcoma and 10 leukemia (cases) and 20% random sample of all other deaths (controls) between 1964−1973 in cohort of *n* = 6,678, exposure assessment by company record for use in work area.

[a]*n* = number of observed cases.
[b]Unless otherwise noted, all studies reported standardized mortality ratios using an external population referent group.
[c]Logistic regression analysis with 15 lag for TCE exposure (Ritz, 1999).
[d]In Morgan et al. (1998) and Garabrant et al. (1988), this category was based on lymphosarcoma and reticulosarcoma.
[e]As presented in Mandel et al. (2006), this category defined as ICD -7, ICDA-8, and ICD-9 codes of 200 and 202.
[f]Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies (1997) Final Report to Hughes Corporation (Communication from Paul A. Cammer, President, Trichloroethylene Issues Group to Cheryl Siegel Scott, U.S. EPA, December 22, 1997).
[g]Estimated relative risks from Blair et al. (1998) from Poisson regression models adjusted for date of hire, calendar year of death and sex.
[h]Estimated relative risks from Radican et al. (2008) from Cox proportional hazard models adjusted for age and sex.
[i]Odds ratio from nested case-control analysis.
[j]Lymphomas, lymphosarcomas, and reticulosarcomas (ICDA8 200-202) in Greenland et al. (1994).
[k]Other lymphatic and hematopoietic tissue neoplasms (Garabrant et al., 1988).

Case-control studies of lymphoma or hairy cell leukemia (a lymphoma according to the WHO's lymphoma classification system [Morton et al., 2007, 2006]) from United States (Connecticut), Germany, Italy, Sweden, and Canada were identified, and are summarized in Table 4-62 (for additional study descriptions, see Appendix B). These studies identified cases from hospital records (Costantini et al., 2008; Hardell et al., 1994; Mester et al., 2006; Miligi et al., 2006; Persson and Fredrikson, 1999; Seidler et al., 2007; Siemiatycki et al., 1991); the Connecticut Tumor Registry (Wang et al., 2009); or the Swedish Cancer Registry (Nordstrom et al., 1998), and population controls. These studies assign potential occupational TCE exposure to cases and controls using self-reported information obtained from a mailed questionnaire (Hardell et al., 1994; Nordstrom et al., 1998; Persson and Fredrikson, 1999) or from direct interview with study subjects, with industrial hygienist ratings of exposure potential and a job exposure matrix (Siemiatycki et al., 1991; Miligi et al., 2006; Seidler et al., 2007; Costantini et al., 2008; Wang et al., 2009). Additionally, three of these large multiple center lymphoma case-control studies examine specific types of NHL (Miligi et al., 2006; Seidler et al., 2007; Wang et al., 2009) or leukemia (Costantini et al., 2008).

Four geographic based studies on lymphoma in adults are summarized in Table 4-63 (for additional study descriptions, see Appendix B) and subjects in three studies are identified based upon their residence in a community where TCE was detected in water serving the community (Vartianen et al., 1993; Cohn et al., 1994; ATSDR, 2006). Both Cohn et al. (1994) and ATSDR (2006) also present estimates for childhood leukemia and these observations are discussed below with other studies reporting on childhood leukemia. A subject is assumed to have a probability of exposure due to residence likely receiving water containing TCE. Most studies do not include statistical models of water distribution networks, which may influence TCE concentrations delivered to a home, nor a subject's ingestion rate to estimate TCE exposure to individual study subjects. ATSDR (2004, 2006) adopts exposure modeling of soil vapor contamination to define study area boundaries and to identify census tracts with a higher probability of exposure to volatile organic solvents without identifying exposure concentrations to TCE and other solvents. In these studies, one level of exposure to all subjects in a geographic area is assigned, although there is some inherent measurement error and misclassification bias because not all subjects are exposed uniformly.

Table 4-62. Case-control studies of TCE exposure and lymphopoietic cancer or leukemia

Population	Cancer type and exposure group	Odds ratio (95% CI)	*n* exposed cases	Reference(s)
Women aged 21−84 in CT, USA	**Non-Hodgkin lymphoma**			Wang et al., 2009
	Any TCE exposure	1.2 (0.9, 1.8)	77	
	Low intensity TCE exposure	1.1 (0.8, 1.6)	64	
	Medium-high intensity TCE exposure	2.2 (0.9, 5.4)	13	
	(*p* for linear trend)	0.06		
	Low probability TCE exposure	1.1 (0.7, 1.8)	43	
	Medium-high probability TCE exposure	1.4 (0.9, 2.4)	34	
	(*p* for linear trend)	0.37		
	Low intensity TCE exposure/low probability	0.9 (0.6, 1.5)	30	
	Low intensity/medium-high probability	1.4 (0.9, 2.4)	34	
	Medium-high intensity/low probability	2.2 (0.9, 5.4)	13	
	Medium-high intensity/medium-high probability		0	
Population in 6 German regions	**Non-Hodgkin lymphoma**			Seidler et al., 2007; Mester et al., 2006
	Any TCE exposure	Not reported		
	Cumulative TCE			
	0 ppm-yrs	1.0	610	
	>0−≤4 ppm-yrs	0.7 (0.4, 1.1)	40	
	4.4−<35 ppm-yrs	0.7 (0.5, 1.2)	32	
	High exposure, >35 ppm-yrs	2.1 (1.0, 4.8)	21	
	(*p* for linear trend)	0.14		
	>35 ppm-yrs, 10 yr lag	2.2 (1.0, 4.9)		

Table 4-62. Case-control studies of TCE exposure and lymphopoietic cancer or leukemia (continued)

Population	Cancer type and exposure group	Odds ratio (95% CI)	*n* exposed cases	Reference(s)
Population in 6 German regions (continued)	**B-cell NHL**			
	Cumulative TCE			
	0 ppm-yrs	1.0	47	
	>0−≤4 ppm-yrs	0.7 (0.5, 1.2)	32	
	4.4−<35 ppm-yrs	0.8 (0.5, 1.3)	27	
	High exposure, >35 ppm-yrs	2.3 (1.0, 5.3)	17	
	(*p* for linear trend)	0.08		
	Diffuse B-cell NHL			
	Cumulative TCE			
	0 ppm-yrs	1.0	139	
	>0−≤4 ppm-yrs	0.5 (0.2, 1.2)	6	
	4.4−<35 ppm-yrs	0.8 (0.3, 1.8)	7	
	High exposure, >35 ppm-yrs	2.6 (0.7, 3.0)	4	
	(*p* for linear trend)	0.03		
	Chronic lymphocytic Leukemia			
	Cumulative TCE			
	0 ppm-yrs	1.0	610	
	>0−≤4 ppm-yrs	1.1 (0.5, 2.4)	10	
	4.4−<35 ppm-yrs	0.7 (0.3, 1.7)	6	
	High exposure, >35 ppm-yrs	0.9 (0.2, 4.5)	2	
	(*p* for linear trend)	0.46		

Table 4-62. Case-control studies of TCE exposure and lymphopoietic cancer or leukemia (continued)

Population	Cancer type and exposure group	Odds ratio (95% CI)	*n* exposed cases	Reference(s)
Population in 8 Italian regions	**Non-Hodgkin lymphoma**			Miligi et al., 2006
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	0.8 (0.5, 1.3)	35	
	Medium/high	1.2 (0.7, 2.0)	35	
	(*p* for linear trend)	0.8		
	Duration exposure, med/high TCE intensity			
	≤15 yr	1.1 (0.6, 2.1)	22	
	>15 yr	1.0 (0.5, 2.6)	12	
	(*p* for linear trend)	0.72		
	Other non-Hodgkin lymphoma			
	TCE exposure intensity, medium/high			
	Small lymphocytic NHL	0.9 (0.4, 2.1)	7	
	Follicular NHL	Not presented	3	
	Diffuse NHL	1.9 (0.9, 3.7)	13	
	Other NHL	1.2 (0.6, 2.4)	11	
	Leukemia			Costantini et al., 2008
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	1.0 (0.5, 1.8)	17	
	Medium/high	0.7 (0.4, 1.5)	11	
	Acute myeloid leukemia			
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	1.0 (0.4, 2.5)	6	
	Medium/high	1.1 (0.5, 2.9)	6	

Table 4-62. Case-control studies of TCE exposure and lymphopoietic cancer or leukemia (continued)

Population	Cancer type and exposure group	Odds ratio (95% CI)	*n* exposed cases	Reference(s)
Population in 8 Italian regions (continued)	**Chronic lymphocytic leukemia**			
	Any TCE exposure	Not reported		
	TCE exposure intensity			
	Very low/low	1.2 (0.5, 2.7)	8	
	Medium/high	0.9 (0.3, 2.6)	4	
Population of Örebro and Linköping, Sweden	**B-cell non-Hodgkin lymphoma**			Persson and Fredrikson, 1999
	Any TCE exposure	1.2 (0.5, 2.4)	16	
Population of Sweden	**Hairy cell lymphoma**			Nordstrom et al., 1998
	Any TCE exposure	1.5 (0.7, 3.3	9	
Population of Umea, Sweden	**Non-Hodgkin lymphoma**			Hardell et al., 1994
	Any exposure to TCE	7.2 (1.3, 42)	4	
Population of Montreal, Canada	**Non-Hodgkin lymphoma**			Siemiatycki et al., 1991
	Any TCE exposure	1.1 (0.6, 2.3)*	6	
	Substantial TCE exposure	0.8 (0.2, 2.5)*	2	

*90% confidence interval.

Table 4-63. Geographic-based studies of TCE and non-Hodgkin lymphoma or leukemia in adults

Population	Exposure group	non-Hodgkin lymphoma		Leukemia		Reference
		Relative risk (95% CI)	*n* exposed cases	Relative risk (95% CI)	*n* exposed cases	
Two study areas in Endicott, NY		0.54 (0.22, 1.12)	7	0.79 (0.34, 1.55)	8	ATSDR, 2006
Residents of 13 census tracts in Redland, CA		1.09 (0.84, 1.38)	111	1.02 (0.74, 1.35)	77	Morgan and Cassady, 2002
Population in New Jersey	Males, maximum estimated TCE concentration (ppb) in municipal drinking water					Cohn et al., 1994
	<0.1	1.00	493	1.00	438	
	0.1−0.5	1.28 (1.10, 1.48)	272	0.85 (0.71, 1.02)	162	
	≥5.0	1.20 (0.94, 1.52)	78	1.10 (0.84, 1.90)	63	
	Females, maximum estimated TCE concentration (ppb) in municipal drinking water					
	<0.1	1.00	504	1.00; 315		
	0.1−0.5	1.02 (0.87, 1.2)	26	1.13 (0.93, 1.37)	156	
	>5.0	1.36 (1.08, 1.70)	87	1.43 (1.43, 1.90)	56	
Population in Finland	Residents of Hausjarvi	0.6 (0.3, 1.1)	14	1.2 (0.8, 1.7)	33	Vartiainen et al., 1993
	Residents of Huttula	1.4 (1.0, 2.0)	13	0.7 (0.4, 1.1)	19	

NHL incidence is statistically significantly elevated in three high-quality studies (3.1, 95% CI: 1.3, 6.1 [Hansen et al., 2001]; 1.5, 95% CI: 1.2, 2.0, subcohort with higher exposure [Raaschou-Nielsen et al., 2003], 2.1, 95% CI: 1.0, 4.8, >35-ppm years cumulative TCE exposure [Seidler et al., 2007]). Two of these incidence studies report statistically significantly associations for all lymphopoietic and hematopoietic cancer, specifically NHL, for subjects with longer employment duration as a surrogate of TCE exposure ($\geq$6.25 year, 4.2, 95% CI: 1.1, 11 [Hansen et al., 2001]; $\geq$5 year, 1.6, 95% CI: 1.1, 2.2, [Raaschou-Nielsen et al., 2003]) and Seidler et al. (2007) report a positive trend with diffuse B-cell NHL and cumulative TCE exposure ($p = 0.03$). Hansen et al. (2001) also examined two other exposure surrogates, cumulative exposure and exposure intensity, with estimated risk larger in low exposure groups than for high exposure groups. A fourth study from Sweden reports a large and imprecise risk with TCE (7.2, 95% CI: 1.3, 42 [Hardell et al., 1994]) based on four exposed cases. High-quality cohort mortality studies and other case-control studies observed a 10 to 50% increased risk between NHL and any TCE exposure (1.2, 95% CI: 0.65, 1.99 [Boice et al., 1999]; 1.36, 95% CI: 0.28, 6.08 [Morgan et al., 1998]; 1.5, 95% CI: 0.7, 3.3 [Nordstrom et al., 1998]; 1.2, 95% CI: 0.5, 2.4 [Persson and Fredrikson, 1999]; 1.36, 95% CI: 0.77, 2.39 [Radican et al., 2008]; 1.1, 95% CI: 0.6, 2.3 [Siemiatycki, 1991]; 1.2, 95% CI: 0.9, 1.8 [Wang et al., 2009]).

Odds ratios are higher for diffuse NHL, primarily a B-cell lymphoma, than for all non-Hodgkin lymphomas in both studies which examine forms of lymphoma (Miligi et al., 2006; Seidler et al., 2007) (see Table 4-63). Observations in the two other studies of B-cell lymphomas (Persson and Fredrikson, 1999; Wang et al., 2009) appear consistent with Miligi et al. (2006) and Seidler et al. (2007). Together, these observations suggest that the associations between trichloroethylene and diffuse NHL are stronger than the associations seen with other forms of lymphoma, and that disease misclassification may be introduced in studies examining trichloroethylene and NHL as a broader category. Mortality observations in other occupational cohorts (Wilcosky et al., 1984; Garabrant et al., 1988; Costa et al., 1989; Greenland et al., 1994; Ritz, 1999; Henschler et al., 1995; Chang et al., 2003; ATSDR, 2004, Boice et al., 2006; Sung et al., 2007) included a risk estimate of 1.0 in 95% confidence intervals; these studies neither add to nor detract from the overall weight of evidence given their lower likelihood for TCE exposure due to inferior exposure assessment approaches, lower prevalence of exposure, lower statistical power, and fewer exposed deaths.

Seven studies presented estimated risks for leukemia and overall TCE exposure (Anttila et al., 1995; Blair et al., 1998 and its update by Radican et al., 2008; Morgan et al., 1998; Boice et al., 1999, 2006; Hansen et al., 2001; Raachou-Nielsen et al., 2003); only three studies also presented estimated risks for a high exposure category (Anttila et al., 1995; Morgan et al.,

1998; Blair et al., 1998). Two case-control studies presented estimated risk for leukemia categories and low or high TCE exposure category (Seidler et al., 2007; Costantini et al., 2008); however, neither study presented estimated risk for overall TCE exposure. Risk estimates in high-quality cohort studies ranged from 0.64 (95% CI: 0.35, 1.18) (Radican et al., 2008) to 2.0 (95% CI: 0.7, 4.44) (Hansen et al, 2001). The largest study, with 82 observed incident leukemia cases, reported a relative risk estimate of 1.2 (95% CI: 0.9, 1.4) (Raaschou-Nielsen et al., 2003). Both case-control studies which examined leukemia risk and TCE exposure are quite limited in statistical power, Costantini et al. (2008) was the largest with 11 exposed cases, and did not provide evidence for an association.

The number of studies of childhood lymphoma including acute lymphatic leukemia and trichloroethylene is much smaller than the number of studies of trichloroethylene and adult lymphomas, and consists of four case-control studies (Costas et al., 2002; Lowengart et al., 1987; McKinney et al., 1991; Shu et al., 1999) and four geographic based studies (Aickin et al., 1992; AZ DHS, 1990, 1995; ATSDR, 2006, 2008; Cohn et al., 1994) (see Table 4-64). An additional publication, focusing on ras mutations, based on one of the case-control studies is also available (Shu et al., 2004). All four case-control studies evaluate maternal exposure, and three studies also examine paternal occupational exposure (Lowengart et al., 1987; McKinney et al., 1991; Shu et al., 2004, 1999). There are relatively few cases with maternal exposure (range 0 to 16) in these case-control studies, and only Shu et al. have a large number (n = 136) of cases with paternal exposure (Shu et al., 2004, 1999). The small numbers of exposed case parents limit examination of possible susceptibility time windows. Overall, evidence for association between parental trichloroethylene exposure and childhood leukemia is not robust or conclusive.

The results from the studies of Costas et al. (2002) and Shu et al. (1999, 2002) suggest a fetal susceptibility to maternal exposure during pregnancy, with relative risks observed for this time period equal or higher than the relative risks observed for periods before conception or after birth (see Table 4-64). The studies by Lowengart et al. (1987) and McKinney et al. (1991) do not provide informative data pertaining to this issue due to the small number (n = <3) of exposed case mothers. A recent update of a cohort study of electronics workers at a plant in Taiwan (Chang et al., 2003, 2005) reported a 4-fold increased risk (3.83; 95% CI: 1.17, 12.55 [Sung et al., 2008]) for childhood leukemia risk among the offspring of female workers employed during the three months before to three months after conception. Exposures at this factory included trichloroethylene, perchloroethylene, and other organic solvents (Sung et al., 2008). The lack of TCE assignment to individual subjects in this study decrease its weight in the overall analysis.

Table 4-64. Selected results from epidemiologic studies of TCE exposure and childhood leukemia

		Relative risk (95% CI)	*n* observed events	Reference(s)
Cohort studies (solvents)				
Childhood leukemia among offspring of electronic workers				Sung et al., 2008
	Nonexposed	1.0[a]	9	
	Exposed pregnancy to organic solvents	3.83 (1.17, 12.55)	6	
Case-control studies				
Children's Cancer Group Study (children ≤15 yrs age)				
	Acute lymphocytic leukemia			
	Maternal occupational exposure to TCE			Shu et al., 1999
	Anytime	1.8 (0.8, 4.1)	15	
	Preconception	1.8 (0.8, 5.2)	9	
	During pregnancy	1.8 (0.5, 6.4)	6	
	Postnatal	1.4 (0.5, 4.1)	9	
	Paternal occupational exposure to TCE			
	Anytime	1.1 (0.8, 1.5)	136	
	Preconception	1.1 (0.8, 1.5)	100	
	During pregnancy	0.9 (0.6, 1.4)	56	
	Postnatal	1.0 (0.7, 1.3)	77	
	K-ras + acute lymphocytic leukemia			Shu et al., 2004
	Maternal occupational exposure to TCE			
	Anytime	1.8 (0.6, 4.8)	5	
	Preconception	2.0 (0.7, 6.3)	4	
	During pregnancy	3.1 (1.0, 9.7)	4	
	Postnatal		0	
	Paternal occupational exposure to TCE			
	Anytime	0.6 (0.3, 1.4)	9	
	Preconception	0.6 (0.3, 1.5)	8	
	During pregnancy	0.3 (0.1, 1.2)	2	
	Postnatal	0.4 (0.1, 1.4)	3	

Table 4-64. Selected results from epidemiologic studies of TCE exposure and childhood leukemia (continued)

		Relative risk (95% CI)	*n* observed events	Reference(s)
Residents of ages ≤19 in Woburn, MA				Costas et al., 2002
	Maternal exposure 2 yrs before conception to diagnosis			
	Never	1.00	3	
	Least	5.00 (0.75, 33.5)	9	
	Most	3.56 (0.51, 24.8)	7	
	(*p* for linear trend)	≥ 0.05		
	Maternal exposure 2 yrs before conception			
	Never	1.00	11	
	Least	2.48 (0.42, 15.2)	4	
	Most	2.82 (0.30, 26.4)	4	
	(*p* for linear trend)	≥0.05		
	Birth to diagnosis			
	Never	1.00	7	
	Least	1.82 (0.31, 10.8)	7	
	Most	0.90 (0.18, 4.56)	5	
	(*p* for linear trend)	≥0.05		
	Maternal exposure during pregnancy			
	Never	1.00	9	
	Least	3.53 (0.22, 58.1)	3	
	Most	14.3 (0.92, 224)	7	
	(*p* for linear trend)	<0.05		
Population ≤14 yrs of age in 3 areas north England, United Kingdom				McKinney et al., 1991
	Acute lymphocytic leukemia and NHL			
	Maternal occupation exposure to TCE			
	Preconception	1.16 (0.13, 7.91)	2	
	Paternal occupational exposure to TCE			
	Preconception	2.27 (0.84, 6.16)	9	
	Periconception and gestation	4.49 (1,15, 21)	7	
	Postnatal	2.66 (0.82, 9.19)	7	
Los Angeles Cancer Surveillance Program				Lowengart et al., 1987
	Acute lymphocytic and nonlymphocytic leukemia, ≤10 yrs of age			
	Maternal occupational exposure to TCE		0	
	Paternal occupational exposure to TCE			
	One year before pregnancy	2.0 ($p = 0.16$)	6/3[b]	
	During pregnancy	2.0 ($p = 0.16$)	6/3[b]	
	After delivery	2.7 (0.64, 15.6)	8/3[b]	

Table 4-64. Selected results from epidemiologic studies of TCE exposure and childhood leukemia (continued)

		Relative risk (95% CI)	*n* observed events	Reference(s)
Geographic based studies				
Two study areas in Endicott, NY				ATSDR, 2006
	Leukemia, ≤19 yrs of age	Not reported	<6	
Population in New Jersey				
	Acute lymphocytic leukemia			
	Maximum estimated TCE concentration (ppb) in municipal drinking water			Cohn et al., 1994
	Males			
	<0.1	1.00	45	
	0.1−0.5	0.91(0.53, 1.57)	16	
	≥5.0	0.54 (0.17, 17.7)	3	
	Females			
	<0.1	1.00	25	
	0.1−0.5	1.85 (1.03, 3.70)	22	
	≥5.0	2.36 (1.03, 5.45)	7	
Resident of Tucson Airport Area, AZ				AZ DHS, 1990, 1995
	Leukemia, ≤19 yrs of age			
	1970−1986	1.48 (0.74, 2.65)	11	
	1987−1991	0.80 (0.31, 2.05)	3	
Resident of West Central Phoenix, AZ				Aickin et al., 1992
	Leukemia, ≤19 yrs of age	1.95 (1.43, 2.63)	38	

[a]Internal referents, live born children among female workers not exposed to organic solvents.
[b]Discordant pairs.

The evidence for an association between childhood leukemia and paternal exposure to solvents is quite strong (Colt and Blair, 1998); however, for studies of TCE exposure, the small numbers of exposed case fathers in two studies (McKinney et al., 1991; Lowengart et al., 1987) and, for all three studies, likelihood of misclassification resulting from a high percentage of paternal occupation information obtained from proxy interviews, limits observation interpretations. Both Lowengart et al. (1987) and McKinney et al. (1991) provide some evidence for a 2- to 4-fold increase of childhood leukemia risk and paternal occupational exposure although the population study of Shu et al. (1999, 2002), with 13% of case father's occupation reported by proxy respondents, does not appear to support the earlier and smaller studies.

The geographic based studies for adult lymphopoietic (see Table 4-63) or childhood leukemias (see Table 4-64) do not greatly contribute to the overall weight of evidence. While

some studies observed statistically significantly elevated risks for NHL or childhood cancer, these studies generally fulfilled only the minimal of evaluation criteria with questions raised about subject selection (Morgan and Cassady, 2002), their use of less sophisticated exposure assessment approaches and associated assumption of an average exposure to all subjects (all studies), and few cases with high level parental exposure (all studies).

4.6.1.2.2. ***Meta-analysis of lymphoma risk.*** Meta-analysis is adopted as a tool for examining the body of epidemiologic evidence on lymphoma and TCE exposure and to identify possible sources of heterogeneity. The meta-analysis of lymphoma examines 16 cohort and case-control studies identified through a systematic review and evaluation of the epidemiologic literature on TCE exposure (Siemiatycki et al., 1991; Axelson et al., 1994; Hardell et al., 1994; Anttila et al., 1995; Greenland et al., 1994; Morgan et al., 1998; Nordstrom et al., 1998; Boice et al., 1999; Persson and Fredrikson, 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Miligi et al., 2006; Seidler et al., 2007; Radican et al., 2008; Wang et al., 2009) and two studies as alternatives (Blair et al., 1998; Boice et al., 2006). These 18 studies of lymphoma and TCE had high likelihood of exposure, were judged to have met, to a sufficient degree, the criteria of epidemiologic design and analysis, and reported estimated risks for overall TCE exposure; 12 of these studies, also, presented estimated lymphoma risk with high level TCE exposure (Siemiatycki et al., 1991; Axelson et al., 1994; Anttila et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Miligi et al., 2006; Seidler et al., 2007; Radican et al., 2008; Wang et al., 2009). Full details of the systematic review, criteria to identify studies for including in the meta-analysis, and meta-analysis methodology and findings are discussed in Appendices B and C.

The meta-analyses of the overall effect of TCE exposure on lymphoma suggest a small, robust, and statistically significant increase in NHL risk. The pooled estimate from the primary random effect meta-analysis (RRp) was 1.23 (95% CI: 1.04, 1.44) (Figure 4-15). This result and its statistical significance were not influenced by individual studies. The result is similarly not sensitive to individual risk ratio estimate selections except that substituting the Zhao et al. (2005) mortality results with the study's incidence results leads to an RRp that is no longer statistically significant of 1.19 (95% CI: 1.00, 1.41).

Meta-analysis of the highest exposure groups, either duration, intensity, or their product, cumulative exposure, results in an RRp of 1.57 (95% CI: 1.27, 1.94), which is greater than the RRp from the overall exposure analysis, and provides additional support for an association between NHL and TCE (Figure 4-16). The highest exposure category groups have a reduced likelihood for exposure misclassification because they are believed to represent a greater

differential TCE exposure compared to people identified with overall TCE exposure. Observation of greater risk associated with higher exposure category compared to overall (typically any versus none) exposure comparison additionally suggests an exposure-response gradient between lymphoma and TCE, although estimation of a level of exposure associated with the pooled or meta-relative risk is not possible.

Low-to-moderate heterogeneity in RRp is observed across the results of the 16 studies in the meta-analysis of the overall effect of TCE, but it was not statistically significant ($p = 0.10$), and no heterogeneity was observed in the meta-analysis of the highest exposure groups. In the overall analysis, difference between cohort and case-control studies could explain much of the observed heterogeneity. In the subgroup analysis, increased risk of lymphoma was strengthened in analysis limited to cohort studies and virtually eliminated in the case-control study analysis. Examination of heterogeneity in cohort and case-control studies separately was not statistically significant in either case although some may be present given that statistical tests of heterogeneity are generally insensitive in cases of minor heterogeneity. In general, sources of heterogeneity are uncertain and may reflect several features known to influence epidemiologic studies. One reason may be differences in exposure assessment and in overall TCE exposure concentration between cohort and case-control studies. Several cohort studies (Anttila et al., 1995; Axelson et al., 1994; Blair et al., 1998; Hansen et al., 2001; Raaschou-Nielsen et al., 2003) adopt exposure assessment approaches that are expected to reduce potential for bias (NRC, 2006). Exposure misclassification bias due to random or measurement error and recall bias a more likely in three case-control studies (Hardell et al., 1994; Nordstrom et al., 1998; Persson and Fredrikson, 1999) with self-reported TCE exposure compared to Siemiatycki (1991), Miligi et al. (2006), Seidler et al. (2007). No heterogeneity was observed in the meta-analysis of the highest exposure groups, providing some evidence of exposure misclassification as a source of heterogeneity in the overall analysis. In addition, a low overall TCE exposure prevalence is anticipated in population case-control studies which would typically assess a large number of workplaces and operations, where exposures are less well defined, and where case and control subjects identified as exposed to TCE probably have minimal contact (NRC, 2006). Observed higher risk ratios with higher exposure categories in NHL case-control studies support exposure differences as a source of heterogeneity.

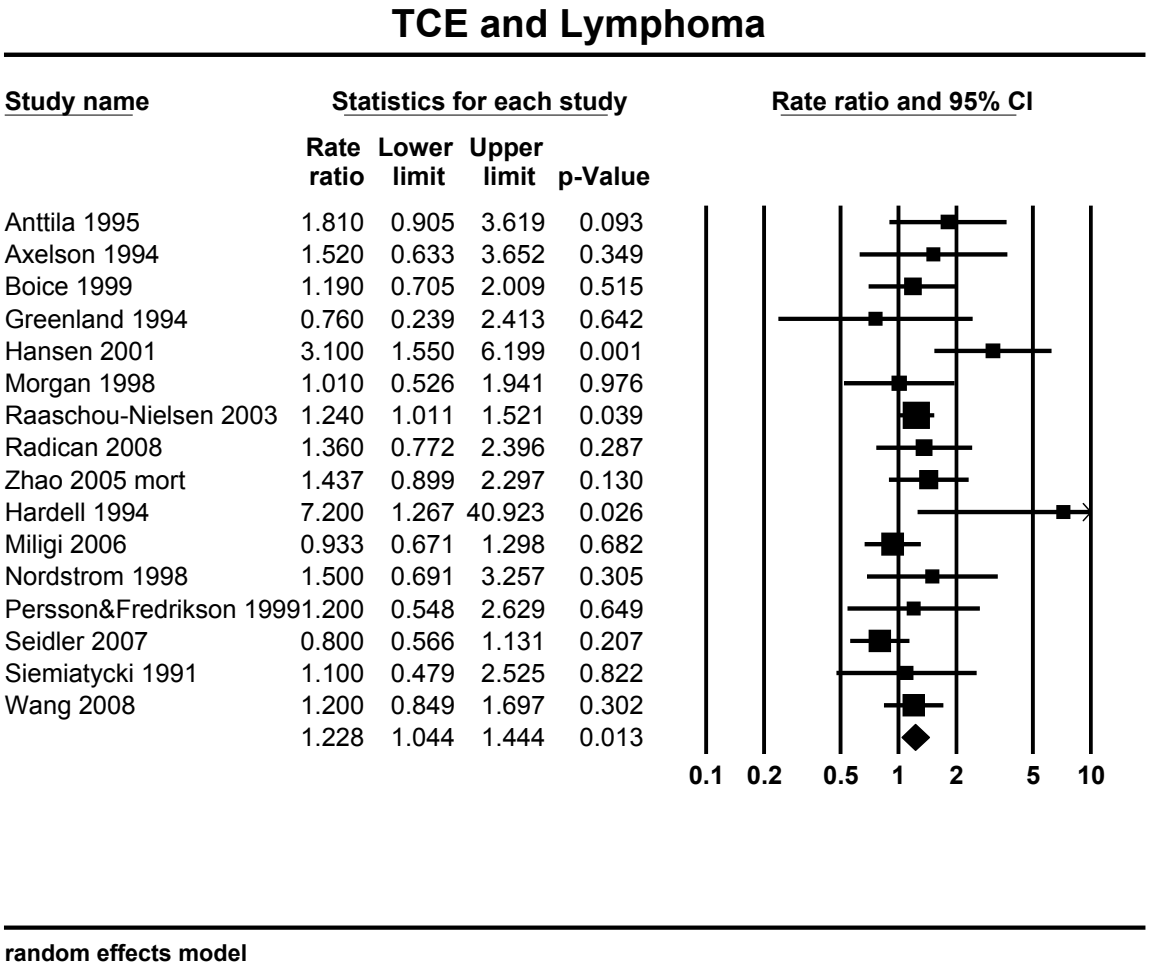

Figure 4-15. Meta-analysis of lymphoma and overall TCE exposure. The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the RRp estimate and the horizontal extremes depict the 95% CI limits.

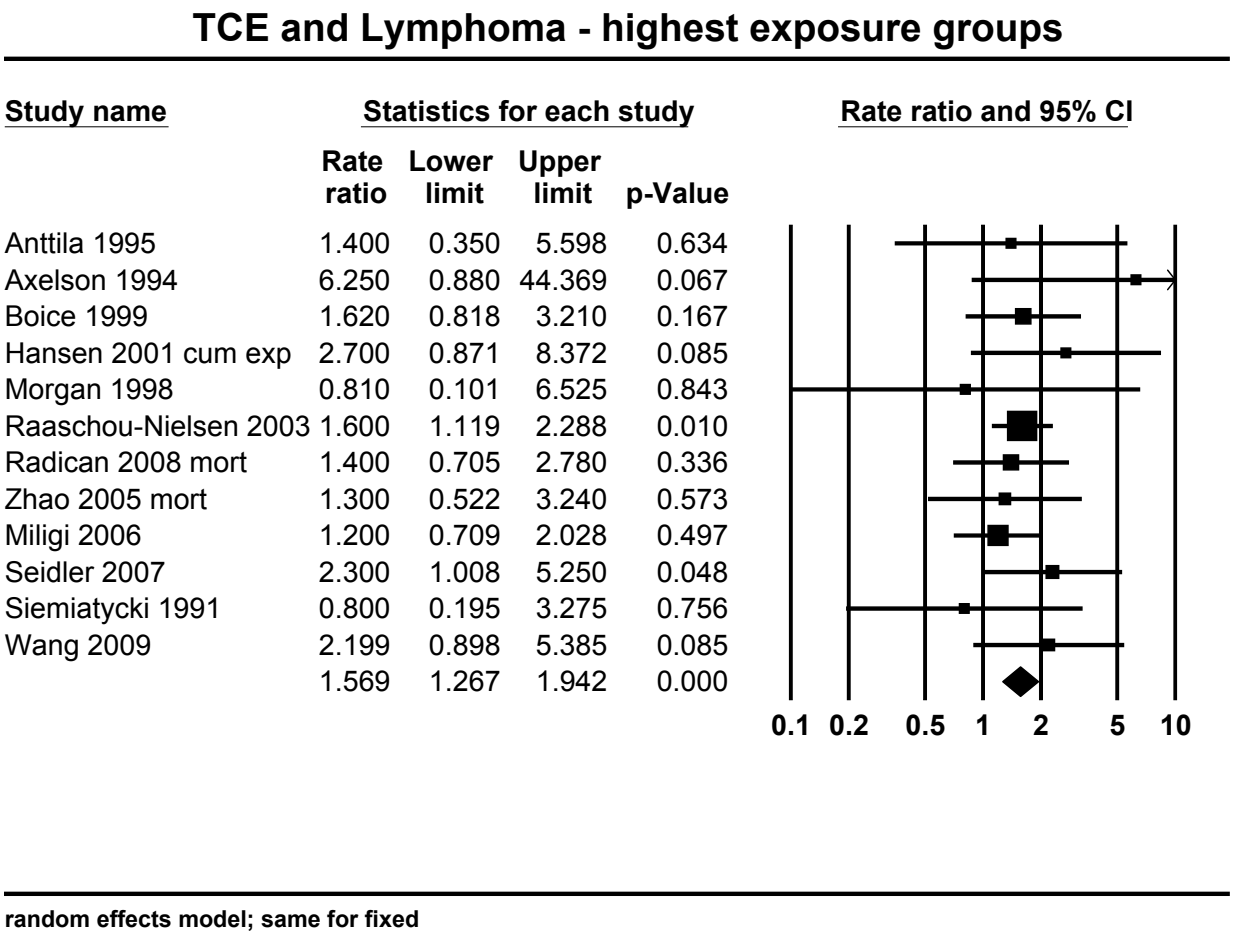

Figure 4-16. Meta-analysis of lymphoma and TCE exposure—highest exposure groups. The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the RRp estimate and the horizontal extremes depict the 95% CI limits.

Diagnostic inaccuracies are likely another source of heterogeneity in the meta-analysis through study differences in lymphoma groupings and in lymphoma classification schemes. All studies include a broad but slightly different group of lymphosarcoma, reticulum-cell sarcoma, and other lymphoid tissue neoplasms (Codes 200 and 202), except Nordstrom et al. (1998) whose case-control study examined hairy cell leukemia, now considered a lymphoma. Cohort studies have some consistency in coding NHL, with NHL defined as lymphosarcoma and reticulum-cell sarcoma (200) and other lymphoid tissue neoplasms (202) using the ICD, Revision 7, 200 and 202—four studies (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001; Raaschou-Nielsen et al., 2003), ICD-Adapted, Revision 8 (Blair et al., 1998), and ICD-7, 8, 9, and 10, per the version in use at the time of death (Morgen et al., 1997, as presented in Mandel et al., 2006; Boice et al., 1999; Radican et al., 2008), as does the case-control study of Siemiatycki (1991) whose coding scheme for NHL is consistent with ICD 9, 200 and 202. Case-control studies, on the other hand, have adopted other classification systems for defining NHL including the NCI Working Formulation (Miligi et al., 2006), WHO (Seidler et al., 2007), Rappaport (Hardell et al., 1994), or else do not identify the classification system for defining NHL (Persson and Fredrikson, 1999).

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 standard error 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.

NRC (2006) deliberations on trichloroethylene commented on two prominent evaluations of the then-current epidemiologic literature using meta-analysis techniques. These studies were by Wartenberg et al. (2000), and by Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations and subsequently published in a paper on NHL (Mandel et al., 2006) and a paper on multiple myeloma and leukemia (Alexander et al., 2006). The NRC found weaknesses in the techniques used in each of these studies, and suggested that U.S. EPA conduct a new meta-analysis of the epidemiologic data on trichloroethylene using objective and transparent criteria so as to improve on the past analyses. U.S. EPA staff conducted their analysis according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control studies. The U.S. EPA analysis of NHL analysis considered a larger number of studies than in the previous analyses (Mandel et al., 2006; Wartenberg et al., 2000), and includes recently published studies (Boice et al., 2006; Miligi et al., 2006; Seidler et al., 2007; Zhao et al., 2005). Despite the weaknesses in Wartenberg et al. (2000), Kelsh (2005) and Mandel et al. (2006), pooled NHL risk for overall TCE exposure in

these analyses is of a similar magnitude as that observed in U.S. EPA's updated analysis (1.5, 95% CI: 0.9, 2.3, Tier 1 incidence; 1.2, 95% CI: 0.9, 1.7, Tier 1 mortality [Wartenberg et al., 2000]; 1.59, 95% CI: 1.21, 2.08, Group I, TCE Subcohorts; 1.39, 95% CI: 0.62, 3.10, case-control studies [Kelsh, 2005; Mandel et al, 2006]).

4.6.2. Animal Studies

The immunosuppressive and immunomodulating potential of TCE has not been fully evaluated in animal models across various exposure routes, over various relevant durations of exposure, across representative life stages, and/or across a wide variety of endpoints. Nevertheless, the studies that have been conducted indicate a potential for TCE-induced immunotoxicity, both following exposures in adult animals and during immune system development (i.e., *in utero* and preweaning exposures).

4.6.2.1. *Immunosuppression*

A number of animal studies have indicated that moderate to high concentrations of TCE over long periods have the potential to result in immunosuppression in animal models, dependant on species and gender. These studies are described in detail below and summarized in Table 4-65.

4.6.2.1.1. ***Inhalation exposures.*** Mature cross-bred dogs (5/group) were exposed to 0-, 200-, 500-, 700-, 1,000-, 1,500-, or 2,000-ppm TCE for 1-hour or to 700 ppm TCE for 4 hours, by tracheal intubation under intravenous sodium pentobarbital anesthesia. An additional group of dogs was exposed by venous injection of 50 mg/kg TCE administered at a rate of 1 mL/minute (Hobara et al., 1984). Blood was sampled pre- and postexposure for erythrocyte and leukocyte counts. Marked, transient decreases in leukocyte counts were observed at all exposure levels 30 minutes after initiation of exposure. At the end of the exposure period, all types of leukocytes were decreased (by 85%); neutrophils were decreased 33%, and lymphocytes were increased 40%. There were no treatment-related changes in erythrocyte counts, hematocrit values, or thrombocyte counts.

Table 4-65. Summary of TCE immunosuppression studies

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[a]	Results	Reference, species/strain sex/number
Inhalation Exposure Studies			
Single 1-h exposure to all dose groups; plus single 4-h exposure at 700 ppm[b] 0, 200, 500, 700, 1,000, 1,500, or 2,000 ppm	LOAEL: 200 ppm	Marked transient ↓ leukocyte counts at all exposure levels 30 min after initiating exposure. At end of exposure, 85% ↓ leukocyte counts (33% ↓ neutrophils, 40% ↓ lymphocytes).	Hobara et al., 1984 Dog, cross-bred, both sexes, 5/group
Single 3-h exposure. Also, 3 h/d on 5 d at lowest dose 0, 2.6, 5.2, 10.6, 25.6, or 48 ppm	NOAEL: 2.6 ppm LOAEL: 5.2 ppm	Challenged with *Streptococcus zooepidemicus* to assess susceptibility to infection and *Klebsiella pneumoniae* to assess bacterial clearance. For single exposure: dose-related sig. ↑ mortality at ≥5.2 ppm over 14 d. Sig. ↓ in bactericidal activity at 10.6 ppm.	Aranyi et al., 1986 Mouse, CD-1 females, 4−5 wk old, approx. 30 mice/group, 5−10 replications; for pulmonary bactericidal activity assay, 17−24 mice/group
Single 3-h exposure, 50−200 ppm[c]		Challenged with *Streptococcus zooepidemicus.* Dose-related ↑ mortality, bacterial antiphagocytic capsule formation, and bacterial survival. Dose-related impairment of alveolar macrophages; increased neutrophils in bronchoalveolar fluid at 3 d postinfection.	Park et al., 1993 (abstract) Mouse, CD-1, (sex and #/group not specified)
4-wk, 6 h/d, 5 d/wk 0, 100, 300, or 1,000 ppm	NOAEL: 300 ppm LOAEL: 1,000 ppm	At 1,000 ppm, 64% ↓ plaque-forming cell assay response.	Woolhiser et al., 2006 Rat, Sprague-Dawley, female, 16/group
Oral Exposure Studies			
Gavage in 10% emulphor, 14 d, daily, 0, 24, or 240 mg/kg/d	LOAEL: 24 mg/kg/d	Sig. ↓ cell-mediated immune response to SRBC at both dose levels.	Sanders et al., 1982 Mouse, CD-1, male, 9−12/group
Drinking water with 1% emulphor, 4−6 months 0, 0.1, 1.0, 2.5, or 5.0 mg/mL	LOAEL: 0.1 mg/kg/d	In females, humoral immunity ↓ at 2.5 and 5 mg/mL TCE, whereas cell-mediated immunity ↓ and bone marrow stem cell colonization ↓ at all four concentrations. The males were relatively unaffected after both 4 and 6 months.	Sanders et al., 1982 Mouse, CD-1, male and female, 7−25/group
Gavage, 14 d, 0, 14.4, or 144 mg/kg/d chloral hydrate	NOAEL: 144 mg/kg/d	No treatment-related effects.	Kauffmann et al., 1982 Mouse, CD-1, male, 12/group

Table 4-65. Summary of TCE immunosuppression studies (continued)

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[a]	Results	Reference, species/strain sex/number
Drinking water, 90 d, 0, 0.07, or 0.7 mg/mL chloral hydrate. (M: 0, 16, or 160 mg/kg/d; F: 0, 18, or 173 mg/kg/d)	NOAEL: 0.07 mg/mL LOAEL: 0.7 mg/mL	Sig. ↓ cell-mediated immune response (plasma hemagglutination titers and spleen antibody-producing cells of mice sensitized to SRBC) in females at 0.7 mg/mL.	Kauffmann et al., 1982 Mouse, CD-1, male and female, 15−20/group
Drinking water, From mating to PND 21 or PND 56, (emulphor conc. not provided) 0 (emulphor), 1, or 10 ppm	LOAEL: 1 ppm	At 10 ppm, ↓ body weight and length at PND 21. IgM antibody response to SRBC challenge suppressed in both ♂ and ♀ pups at 10 ppm, and ♂ pups at 1 ppm, ↓ in splenic CD4+CD8-T-cells. At 56 PND, striking ↑ in natural killer cell activity seen at both doses.	Adams et al., 2003 (abstract) Mouse, B6C3F1, both sexes, numbers of pups not stated
Drinking water, from GD 0 to 3 or 8 wks of age, 0, 1,400, or 14,000 ppb	LOAEL: 1,400 ppb	Suppressed PFC responses in both sexes and ages at 14,000 ppb, in males at both ages at 1,400 ppb, and in females at 8 wks at 1,400 ppb. Numbers of spleen B220+ cells ↓ at 3-wks at 14,000 ppb. Pronounced ↑ thymus T-cell populations at 8 wks.	Peden-Adams et al., 2006 Mouse, B6C3F1, dams and both sexes offspring, 5 litters/group; 5−7 pups/group at 3 wks; 4−5 pups/sex/group at 8 wks
Drinking water, from GD 0 to 7−8 wks of age; 0, 0.5, or 2.5 mg/mL	LOAEL: 0.5 mg/mL	At 0.5 mg/mL: Sig ↓ postweaning weight; sig.↑ IFNγ produced by splenic CD4+ cells at 5−6 wks; sig ↓ splenic CD8+ and B220+ lymphocytes; sig.↑ IgG2a and histone; sig. altered CD4-/CD8- and CD4+/CD8+ thymocyte profile At 2.5 mg/mL: Sig ↓ postweaning weight; sig.↑ IFNγ produced by splenic CD4+ and CD8+ cells at 4−5 and 5−6 wks; sig ↓ splenic CD4+, CD8+, and B220+ lymphocytes; sig. altered CD4+/CD8+ thymocyte profile.	Blossom and Doss, 2007 Mouse, MRL +/+, dams and both sexes offspring, 3 litters/group; 8−12 pups/group
Drinking water, from GD 0 to PND 42; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg/d; offspring PND 24−42 dose = 31.0 mg/kg/d	LOAEL: 0.1 mg/mL	At 0.1 mg/mL: at PND 20, sig. ↑ thymocyte cellularity and distribution, associated with sig. ↑ in thymocyte subset distribution; sig. ↑ reactive oxygen species generation in total thymocytes; sig. ↑ in splenic CD4+ T-cell production of IFN-γ and IL-2 in females and TNF-α in males at PND 42.	Blossom et al., 2008 Mouse, MRL +/+, dams and both sexes offspring, 8 litters/group; 3−8 pups/group
Drinking water, from GD 0 to 12 months of age; 0 (1% emulphor), 1,400, or 14,000 ppb	LOAEL: 1,400 ppb	At 1,400 ppb: splenic CD4-/CD8- cells sig.↑ in females; thymic CD4+/CD8+ cells sig. ↓ in males; 18% ↑ in male kidney weight. At 14,000 ppb: thymic T-cell subpopulations (CD8+, CD4/CD8-, CD4+) sig. ↓ in males.	Peden-Adams et al., 2008 (in press) Mouse, MRL +/+, dams and both sexes offspring, unknown # litters/group, 6−10 offspring/sex/group

Table 4-65. Summary of TCE immunosuppression studies (continued)

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[a]	Results	Reference, species/strain sex/number
Intraperitoneal Injection Exposure Studies			
3 d, single daily injection, 0, 0.05, 0.5, or 5 mmol/kg/day	NOAEL: 0.05 mmol/kg/day LOAEL: 0.5 mmol/kg/day	↓ natural killer cell activity at 0.5 and 5 mmol/kg/day. ↓ splenocyte counts at 5 mmol/kg/day.	Wright et al., 1991 Rat, Sprague-Dawley
3 d, single daily injection, 0 or 10 mmol/kg/day	LOAEL: 10 mmol/kg/day	↓ natural killer cell activity and ↓ spleen weights at 10 mmol/kg/day.	Wright et al., 1991 Mouse, B6C3F1

[a]NOAEL and LOAEL are based upon reported study findings.
[b]Inhalation, tracheal intubation under anesthesia.
[c]Exact dose levels not specified.

↓, ↑ = decreased, increased; sig. = statistically significant.

In a study that examined the effects of a series of inhaled organic chemical air contaminants on murine lung host defenses, Aranyi et al. exposed female CD-1 mice to single 3-hour exposures of TCE at time-weighted concentrations of 0, 2.6, 5.2, 10.6, 25.6, or 48 ppm (Aranyi et al., 1986). Additionally, at the dose at which no adverse treatment-related effect occurred with a single exposure (i.e., 2.6 ppm), a multiple exposure test (5 days, 3 hours/day) was conducted. Susceptibility to Streptococcus zooepidemicus aerosol infection and pulmonary bactericidal activity to inhaled Klebsiella pneumoniae were evaluated. There was a significant ($p < 0.0001$) treatment by concentration interaction for mortality, with the magnitude of the effect increasing with concentration. A significant ($p < 0.0001$) treatment by concentration interaction was also found for bactericidal activity. Single 3-hour exposures at 10.6, 25.6, and 48 ppm resulted in significant increases in mortality, although increases observed after single exposures at 5.2 or 2.6 ppm or five exposures at 2.6 ppm were not significant. Pulmonary bactericidal activity was significantly decreased after a single exposure at 10.6 ppm, but single exposures to 2.6 or 5.2 ppm resulted in significant increases.

In a host-resistance assay, CD-1 mice (sex and number/group not specified) exposed to TCE by inhalation for 3 hours at 50−200 ppm were found to be more susceptible to increased infection following challenge with Streptococcus zooepidemicus administered via aerosol (Park et al., 1993). Dose-related increases in mortality, bacterial antiphagocytic capsule formation, and bacterial survival were observed. Alveolar macrophage phagocytosis was impaired in a dose-responsive manner, and an increase in neutrophils in bronchoalveolar lavage fluid was observed in exposed mice 3 days post infection.

A guideline (OPPTS 870.3800) 4-week inhalation immunotoxicity study was conducted in female Sprague-Dawley rats (Woolhiser et al., 2006). The animals (16/group) were exposed to TCE at nominal levels of 0, 100, 300, or 1,000 ppm for 6 hours/day, 5 days/week. Effects on the immune system were assessed using an antigen response assay, relevant organs weights, histopathology of immune organs, and hematology parameters. Four days prior to study termination, the rats were immunized with sheep red blood cells (SRBC), and within 24 hours following the last exposure to TCE, a plaque forming cell assay was conducted to determine effects on splenic anti-SRBC IgM response. Minor, transient effects on body weight and food consumption were noted in treated rats for the first 2 weeks of exposure. Mean relative liver and kidney weights were significantly ($p = 0.05$) increased at 1,000 ppm as compared to control, while lung, spleen, and thymus weights were similar to control. No treatment-related effects were observed for hematology, WBC differential counts, or histopathological evaluations (including spleen, thymus, and lung-associated lymph nodes). At 1,000 ppm, rats demonstrated a 64% decrease in plaque forming cell assay response. Lactate dehydrogenase, total protein

levels, and cellular differentiation counts evaluated from bronchoalveolar lavage (BAL) samples were similar between control and treated groups. A phagocytic assay using BAL cells showed no alteration in phagocytosis, although these data were not considered fully reliable since (1) the number of retrieved macrophage cells was lower than expected and pooling of samples was conducted and (2) samples appear to have been collected at 24 hours after the last exposure (rather than within approximately 2 hours of the last exposure), thereby allowing for possible macrophage recovery. The NOAEL for this study was considered by the study authors to be 300 ppm, and the LOAEL was 1,000 ppm; however, the effect level may have actually been lower. It is noted that the outcome of this study does not agree with the studies by Aranyi et al. (1986) and Park et al. (1993), both of which identified impairment of macrophage phagocytic activity in BAL following inhalation TCE exposures.

4.6.2.1.2. ***Oral exposures.*** In a study by Sanders et al., TCE was administered to male and female CD-1 mice for 4 or 6 months in drinking water at concentrations of 0, 0.1, 1, 2.5, or 5 mg/mL (Sanders et al., 1982). In females, humoral immunity was suppressed at 2.5 and 5 mg/mL, while cell-mediated immunity and bone marrow stem cell activity were inhibited at all dose levels. Male mice were relatively unaffected either at 4 or 6 months, even though a preliminary study in male CD-1 mice (exposed to TCE for 14 days by gavage at 0, 24, or 240 mg/kg/d) had demonstrated a decrease in cell-mediated immune response to SRBC in male mice at both treatment levels.

A significant decrease in humoral immunity (as measured by plasma hemagglutination titers and the number of spleen antibody producing cells of mice sensitized to sheep erythrocytes) was observed by Kaufmann et al. (1982) in female CD-1 mice (15−20/group) following a 90-day drinking water exposure to 0, 0.07, or 0.7 mg/mL (equivalent to 0, 18, or 173 mg/kg) chloral hydrate, a metabolite of TCE. Similar responses were not observed in male CD-1 mice exposed for 90 days in drinking water (at doses of 0, 16, or 160 mg/kg/d), or when administered chloral hydrate by gavage to 12/group for 14 days at 14.4 or 144 mg/kg/d.

The potential for developmental immunotoxicity was assessed in B6C3F1 mice administered TCE in drinking water at dose levels of 0, 1,400 or 14,000 ppb from gestation day (GD) 0 to either 3 or 8 weeks of age (Adams et al., 2003 [preliminary data]; Peden-Adams et al., 2006). At 3 and 8 weeks of age, offspring lymphocyte proliferation, NK cell activity, SRBC-specific IgM production (PFC response), splenic B220+ cells, and thymus and spleen T-cell immunophenotypes were assessed. Delayed-typed hypersensitivity and autoantibodies to ds-DNA were evaluated in offspring at 8 weeks of age. Observed positive responses consisted of suppressed PFC responses in males at both ages and both TCE treatment levels, and in females at

both ages at 14,000 ppb and at 8 weeks of age at 1,400 ppb. Spleen numbers of B220+ cells were decreased in 3-week old pups at 14,000 ppb. Pronounced increases in all thymus T-cell subpopulations (CD4+, CD8+, CD4+/CD8+, and CD4-/CD8-) were observed at 8 weeks of age. Delayed hypersensitivity response was increased in 8-week old females at both treatment levels and in males at 14,000 ppb only. No treatment-related increase in serum anti-ds-DNA antibody levels was found in the offspring at 8 weeks of age.

In a study designed to examine potential susceptibility of the young (Blossom and Doss, 2007), TCE was administered to groups of pregnant MRL +/+ mice in drinking water at occupationally-relevant levels of 0, 0.5, or 2.5 mg/mL. A total of 3 litters per treatment group were maintained following delivery (i.e., a total of 11 pups at 0 mg/mL TCE, 8 pups at 0.5 mg/mL TCE, and 12 pups at 2.5 mg/mL TCE), and TCE was continuously administered to the offspring until young adulthood (i.e., 7−8 weeks of age). Although there were no effects on reproduction, offspring postweaning body weights were significantly decreased in both treated groups. Additionally, TCE exposure was found to modulate the immune system following developmental and early life exposures. Decreased spleen cellularity and reduced numbers of CD4+, CD8+, and B220+ lymphocyte subpopulations were observed in the postweaning offspring. Thymocyte development was altered by TCE exposures, as evidenced by significant alterations in the proportions of double-negative subpopulations and inhibition of *in vitro* apoptosis in immature thymocytes. TCE was also shown to induce a dose-dependent increase in CD4+ and CD8+ T-lymphocyte IFNγ in peripheral blood by 4−5 weeks of age, although these effects were no longer observed at 7−8 weeks of age. Serum anti-histone autoantibodies and total IgG_{2a} were significantly increased in treated offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys at sacrifice.

This increase in T-cell hyperactivity was further explored in a study by Blossom et al. (2008). In this study, MRL +/+ mice were treated in the drinking water with 0 or 0.1 mg/mL TCE. Based on drinking water consumption data, average maternal doses of TCE were 25.7 mg/kg/d, and average offspring (PND 24-42) doses of TCE were 31.0 mg/kg/d. Treatment was initiated at the time of mating, and continued in the females (8/group) throughout gestation and lactation. Pups were weaned at PND 24, and the offspring were continued on drinking water treatment in a group-housed environment until study termination (PND 42). Subsets of offspring were sacrificed at PND 10 and 20, at which time developmental and functional endpoints in the thymus were evaluated (i.e., total cellularity, CD4+/CD8+ ratios, CD24 differentiation markers, and double-negative subpopulation counts). Indicators of oxidative stress were measured in the thymus at PND 10 and 20, and in the brain at PND 42. Mitogen-induced intracellular cytokine production by splenic CD4+ and CD8+ T-cells was evaluated in juvenile mice and brain tissue

was examined at PND 42 for evidence of inflammation. Behavioral testing was also conducted; these methods and results are described in Section 4.3. TCE treatment did not affect reproductive capacity, parturition, or ability of dams to maintain litters. The mean body weight of offspring was not different between the control and treated groups. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at PND 20 (sexes combined). TCE treatment also appeared to promote T-cell differentiation and maturation at PND 42, and *ex vivo* evaluation of cultured thymocytes indicated increased reactive oxygen species (ROS) generation. Evaluation of peripheral blood indicated that splenic CD4+ T-cells from TCE-exposed PND 42 mice produced significantly greater levels of IFN-γ and IL-2 in males and TNF-α in both sexes. There was no effect on cytokine production on PND 10 or 20. The dose of TCE that resulted in adverse offspring outcomes in this study (i.e., 0.1 mg/mL, equivalent to 25.7−31.0 mg/kg/d) is comparable to that which has been previously demonstrated to result in immune system alterations and autoimmunity in adult MRL +/+ mice (i.e., 0.1 mg/mL, equivalent to 21 mg/kg/d; Griffin et al., 2000b).

Another study that examined the effects of developmental exposure to TCE on the MRL+/+ mouse was conducted by Peden-Adams et al. (2008). In this study, MRL/MpJ (i.e., MRL +/+) mice (unspecified number of dams/group) were exposed to TCE (solubilized with 1% emulphor) in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continuing until the offspring were 12 months of age. TCE concentrations in the drinking water were reported to be analytically confirmed. Endpoints evaluated in offspring at 12 months of age included final body weight; spleen, thymus, and kidney weights; spleen and thymus lymphocyte immunophenotyping (CD4 or CD8); splenic B-cell counts; mitogen-induced splenic lymphocyte proliferation; serum levels of autoantibodies to dsDNA and glomerular antigen (GA), periodically measured from 4 to 12 months of age; and urinary protein measures. Reported sample sizes for the offspring measurements varied from 6 to 10 per sex per group; the number of source litters represented within each sample was not specified. The only organ weight alteration was an 18% increase in kidney weight in the 1,400 ppb males. Splenic CD4-/CD8- cells were altered in female mice (but not males) at 1,400 ppm only. Splenic T-cell populations, numbers of B220+ cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8+, CD4-/CD8-, and CD4+) were significantly decreased in male but not female mice following exposure to 14,000-ppb TCE, and CD4+/CD8+ cells were significantly reduced in males by treatment with both TCE concentrations. Autoantibody levels (anti-dsDNA and anti-GA) were not increased in the offspring over the

course of the study, indicating that TCE did not contribute to the development of autoimmune disease markers following developmental exposures that continued into adult life.

Overall, the studies by Peden-Adams et al. (2006, 2008 in press), Blossom and Doss (2007), and Blossom et al. (2008), which examined various immunotoxicity endpoints following exposures that spanned the critical periods of immune system development in the rodent, were generally not designed to assess issues such as posttreatment recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures.

4.6.2.1.3. ***Intraperitoneal administration.*** Wright et al. reported that following 3 days of single intraperitoneal injections of TCE in Sprague-Dawley rats at 0, 0.05, 0.5, or 5 mmol/kg/day and B6C3F1 mice at 0 or 10 mmol/kg/day, NK cell activity was depressed in the rats at the mid- and high-dose levels, and in the mice at the high dose level (Wright et al., 1991). Also at the highest dose levels tested, decreased splenocyte counts and relative spleen weight were observed in the rats and mice, respectively. *In vitro* assays demonstrated treatment-related decreases in splenocyte viability, inhibition of lipopolysaccharide-stimulated lymphocyte mitogenesis, and inhibited NK cell activity suggesting the possibility that compromised immune function may play a role in carcinogenic responses of experimental animals treated with TCE.

4.6.2.2. ***Hypersensitivity***

Evidence of a treatment-related increase in delayed hypersensitivity response has been observed in guinea pigs following dermal exposures with TCE and in mice following exposures that occurred both during development and postnatally (see Table 4-66).

In a modified guinea pig maximization test, Tang et al. evaluated the contact allergenicity potential of TCE and three metabolites (trichloroacetic acid, trichloroethanol, and chloral hydrate) in 4 animals (FMMU strain, sex not specified) per group (Tang et al., 2002). Edema and erythema indicative of skin sensitization (and confirmed by histopathology) were observed. Sensitization rates were reported to be 71.4% for TCE and 58.3% for trichloroacetic acid, as compared to a reference positive control response rate (i.e., 100% for 2,4-dinitrochlorobenzene). In this study, the mean response scores for TCE, trichloroacetic acid, and 2,4-dinitrochlorobenzene were 2.3, 1.1, and 6.0, respectively. TCE was judged to be a strong allergen and TCA was a moderate allergen, according to the criteria of Magnusson and Kligman (Magnusson and Kligman, 1969). Trichloroethanol and chloral hydrate were not found to elicit a dermal hypersensitivity response.

Table 4-66. Summary of TCE hypersensitivity studies

Exposure route/vehicle, duration, dose	NOAEL; LOAEL[a]	Results	Reference, species/strain sex/number
Induction by single intradermal injection, then challenge by dermal application at 21 d 0 or 0.1 mL induction; 0 or 0.2 mL challenge TCE, TCA, TCOH, and chloral hydrate		Edema and erythema (confirmed by histopathology) indicative of skin sensitization for TCE (strong sensitizer) and TCA (moderate sensitizer)	Tang et al., 2002 Guinea pig, FMMU strain, sex not specified, 4/group
Intradermal injection, 0, 167, 500, 1,500, or 4,500 mg/kg Dermal patch, 0 or 900 mg/kg Hypersensitivity: total dose from induction through challenge <340 mg/kg	Intradermal NOAEL: 500 mg/kg Intradermal LOAEL: 1,500 mg/kg Dermal patch NOAEL: 900 mg/kg	Intradermal injection: At 1,500 mg/kg: Sig. ↑ AST; at 4,500 mg/kg, sig. ↑ ALT and AST, sig. ↓ total protein and globulin; fatty degeneration of liver Dermal patch: no effects of treatment Hypersensitivity: sensitization rate of 66% (strong sensitizer), with edema and erythema; sig. ↑ ALT, AST, and lactate dehydrogenase; sig. ↑ relative liver weight; sig. ↓ albumin, IgA, and GGT; hepatic lesions (ballooning changes)	Tang et al., 2008 Guinea pig, FMMU strain, female, 5−6/group for intradermal/dermal patch study, 10/group for hypersensitivity study, female
Drinking water, from GD 0 to 8 wks of age 0, 1,400, or 14,000 ppb	LOAEL: 1,400 ppb	Sig. ↑ swelling of foot pad in females at 1,400 and in both sexes at 14,000 ppb	Peden-Adams et al., 2006 Mouse, B6C3F1, both sexes, 5 litters/group; 4−5 pups/sex/group at 8 wks[b]

[a]NOAEL and LOAEL are based upon reported study findings.
[b]Subset of immunosuppression study.

↓, ↑ = decreased, increased, sig. = statistically significant.

Immune-mediated hepatitis associated with dermal hypersensitivity reactions in the guinea pig following TCE exposures was characterized by Tang et al. (2008). In this study, FMMU strain female guinea pigs (5−6/group) were treated with intradermal injection of 0, 167, 500, 1,500, or 4,500 mg/kg TCE or with a dermal patch containing 0 or 900 mg/kg TCE and sacrificed at 48 hours posttreatment. At the intradermal dose of 1,500 mg/kg, a significant increase ($p < 0.05$) in serum AST level was observed. At 4,500 mg/kg, significantly ($p < 0.01$) increased ALT and AST levels were reported, and total protein and globulin decreased significantly ($p < 0.05$). Histopathological examination of the liver revealed fatty degeneration, hepatic sinusoid dilation, and inflammatory cell infiltration. No changes were observed at the intradermal doses of 500 mg/kg or below, or the dermal patch dose of 900 mg/kg. A Guinea Pig Maximization Test was also conducted according to the procedures of Magnusson and Kligman on 10 FMMU females/group, in which the total TCE dosage from induction through challenge phases was below 340 mg/kg. TCE treatment resulted in dermal erythema and edema, and the sensitization rate was 66% (i.e., classified as a strong sensitizer). Significant increases ($p < 0.05$) in ALT, AST, lactate dehydrogenase, and relative liver weight, and significant decreases ($p < 0.05$) in albumin, IgA, and γ-glutamyl transpeptidase (GGT) were observed. Additionally, hepatic lesions (diffuse ballooning changes without lymphocyte infiltration and necrotic hepatocytes) were noted. It was concluded that TCE exposure to guinea pigs resulted in delayed type hypersensitivity reactions with hepatic injury that was similar to occupational medicamentosa-like dermatitis disorders observed in human occupational studies.

Also, as indicated in Section 4.6.2.1.2 above, in a developmental immunotoxicity-type study in B6C3F1 mice, administration of TCE in drinking water at dose levels of 0, 1,400, or 14,000 ppb from gestation Day 0 through to 8 weeks of age resulted in an increased delayed hypersensitivity response in 8-week old female offspring at both treatment levels and in males at the high dose of 14,000 ppb (Peden-Adams et al., 2006).

In an *in vitro* study that evaluated a number of chlorinated organic solvents, nonpurified rat peritoneal mast cells (NPMC) and rat basophilic leukemia (RBL-2H3) cells were sensitized with anti-dinitrophenol (DNP) monoclonal IgE antibody and then stimulated with DNP-conjugated bovine serum albumin plus TCE (Seo et al., 2008). TCE enhanced antigen-induced histamine release from NPMC and RBL-2H3 cells in a dose-related manner, and increased IL-4 and TNF-α production from the RBL-2H3 cells. In an *in vivo* study, i.p.-injected TCE was found to markedly enhance passive cutaneous anaphylaxis reaction in antigen-challenged rats. These results suggest that TCE increases histamine release and inflammatory mediator production from antigen-stimulated mast cells via the modulation of immune responses; TCE exposure may lead to the enhancement of allergic disease through this response.

4.6.2.3. *Autoimmunity*

A number of studies have been conducted to examine the effects of TCE exposure in mouse strains (i.e., MRL +/+, MRL –lpr, or NZB × NZW) which are all known to be genetically susceptible to autoimmune disease. The studies have demonstrated the potential for TCE to induce autoimmune disease (as demonstrated in Table 4-67 which summarizes those studies which assessed serology, *ex vivo* assays of cultured splenocytes, and/or clinical or histopathology). These and other studies conducted in susceptible mouse strains have proven to be useful tools in exploring various aspects of the mode of action for this response.

Khan et al. used the MRL +/+ mouse model to evaluate the potential for TCE and one of its metabolites, dichloroacetyl chloride (DCAC) to elicit an autoimmune response (Khan et al., 1995). Female mice (4−5/group) were dosed by intraperitoneal injection with 10 mmol/kg TCE or 0.2 mmol/kg DCAC every 4th day for 6 weeks and then sacrificed. Spleen weights and IgG were increased. ANA and anti-ssDNA antibodies were detected in the serum of TCE- and DCAC-treated mice; anti-cardiolipin antibodies were detected in the serum of DCAC-treated mice. A greater magnitude of response observed with DCAC treatment suggested that the metabolite may be important to the mechanism of TCE-induced autoimmunity.

Other studies in female MRL +/+ mice (8/group) examined exposure via drinking water. In one of these studies, mice were treated with 2.5 or 5.0 mg/mL (455 or 734 mg/kg/d) TCE in drinking water for up to 22 weeks (Gilbert et al., 1999; Griffin et al., 2000a). Serial sacrifices were conducted at Weeks 4, 8, and 22. Significant increases in ANA and total serum immunoglobulin were found at 4 weeks of TCE treatment (indicating an autoimmune response), but not at 32 weeks. Increased expression of the activation marker C44 on splenic CD4+ cells was observed at 32 weeks. In addition, at 4 and 32 weeks, splenic T-cells from treated mice secreted more IFN-γ than control T-cells (significant at 0.5 and 2.5 mg/mL), consistent with a Th1 immune or inflammatory response. By 22 weeks of TCE treatment, a specific immune serum antibody response directed against dichloroacetylated proteins was activated in hepatic tissues, indicating the presence of protein adducts. There was a slight but statistically significant increase in serum alanine aminotransferase levels at 32 weeks at 0.5 mg/mL. Histopathological evaluation at 32 weeks revealed extensive hepatic lymphocytic cell infiltration at 0.5 and 2.5 mg/mL; all treated groups contained significantly more hepatocyte reactive changes (i.e., presence of multinucleated hepatocytes, variations in hepatocyte morphology, and hepatocytes in mitosis) than controls.

Table 4-67. Summary of autoimmune-related studies of TCE and metabolites in mice and rats (by sex, strain, and route of exposure)[a]

Nunber/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	*Ex vivo* assays of cultured splenocytes	Clinical and histopathology	
Autoimmune-prone: Female MRL +/+ Mice, Drinking Water					
8 per group, 0, 2.5, or 5 mg/mL TCE (average 0, 455, or 734 mg/kg/d), 4, 8, or 22 wks	LOAEL: 2.5 mg/mL	Increased ANA at 4 and 8 wks, no difference between groups at 22 wks	Increased activated CD4+ T-cells and IFN-γ secretion across doses at 4 wks, these effects were reversed at 22 wks; decreased IL-4 secretion (4 and 22 wks)	No evidence of liver or renal damage, based on serum alanine aminotransferase, sorbitol dehydrogenase, and blood urea nitrogen.	Griffin et al. (2000a)
8 per group, 0, 0.1, 0.5, or 2.5 mg/mL TCE (0, 21, 100, or 400 mg/kg/d), 4 or 32 wks	LOAEL: 0.1 mg/mL	Increased ANA in all treated groups at 4 wks, but not at 32 wks	Increased activated CD4+ T-cells (32 wks), IFN-γ secretion (4 and 32 wks), no effect on IL-4 secretion	Extensive hepatic mononuclear cellular infiltrate in 0.5 and 2.5 mg/mL groups, and hepatocyte reactive changes in all treated groups at 32 wks.	Griffin et al. (2000b)
6−8 per group, 0, 0.1, or 0.9 mg/mL trichloroacetaldehyde hydrate (0, 24, or 220 mg/kg/d) or trichloroacetic acid (0, 27, or 205 mg/kg/d), 4 wks	LOAEL: 0.1 mg/mL	Increased ANA and anti-histone antibodies at 0.9 mg/mL trichloroacetaldehyde hydrate[c]	Increased activated CD4+ T-cells at 0.1 and 0.9 g/mL doses of both metabolites. At 0.9 mg/mL, increased IFN-γ secretion, no effect on IL-4 secretion	No evidence of liver of kidney damage, based on serum alanine aminotransferase, liver and kidney histology..	Blossom et al. (2004)
8 per group, 0, 0.1, 0.3, or 0.9 mg/mL trichloroacetaldehyde hydrate (0, 13, 46, or 143 mg/kg/d), 40 wks	LOAEL: 0.9 mg/mL	Slightly suppressed anti-ssDNA, anti-dsDNA, and anti-histone antibody expression; differences not statistically significant	Increased activated CD4+ T-cells and increased INF-γ secretion, no effect on IL-4 secretion	Diffuse alopecia, skin inflammation and ulceration, mononuclear cell infiltration, mast cell hyperplasia, dermal fibrosis. Statistically significant increase at 0.9 mg/mL dose group, but also increased at lower doses. No liver or kidney histopathology effects seen.	Blossom et al. (2007)

Table 4-67. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	*Ex vivo* assays of cultured splenocytes	Clinical and histopathology	
5 per group, 0 or 0.5 mg/mL TCE (mean 60 µg/g-d), 48 wks	LOAEL: 0.5 mg/mL	Increased ANA after 24 wks but not statistically significant	Increased INF-γ secretion after 36 wks but not statistically significant	Hepatic necrosis; hepatocyte proliferation; leukocyte infiltrate in the liver, lungs, and kidneys; no difference in serum aminotransferase liver enzymes	Cai et al. (2008)
Autoimmune-prone: male and female offspring MRL +/+ mice, drinking water					
3 litters/group, 8−12 offspring/group; 0, 0.5, or 2.5 mg/mL, GD 0 to 7−8 wks of age	LOAEL: 0.5 mg/mL	Increased anti-histone antibodies and total IgG_{2a} in treated groups	Dose-dependant increase in IFN-γ secretion at 4−5 wks of age but not 7−8 wks of age	No histopathological effects in liver or kidneys	Blossom and Doss (2007)
8 litters/group, 8−12 offspring/group; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg/d; offspring PND 24-42 dose = 31.0 mg/kg/d; GD 0 to PND 42	LOAEL: 0.1 mg/mL	Not evaluated	Increased IFN-γ and IL-2 in females, increased TNF- α in both sexes	Not evaluated	Blossom et al. (2008)
Unknown # litters/group, 6−10 offspring/sex/group; 0 (1% emulphor), 1400, or 14,000 ppb; GD 0 to 12 months of age	NOAEL: 1,400 ppb	No increase in autoantibody levels	Not evaluated	Not evaluated	Peden-Adams et al. (2008)

Table 4-67. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	*Ex vivo* assays of cultured splenocytes	Clinical and histopathology	
Autoimmune-prone: Female MRL +/+ Mice, Intraperitoneal Injection					
4–5 per group, 0 (corn oil), 10 mmol/kg TCE, or 0.2 mmol/kg dichloroacetyl chloride, every 4th day for 6 wks	LOAEL: 10 mmol/kg TCE, 0.2 mmol/kg dichloroacetyl chloride	In both groups, increased ANA and anti-ssDNA antibodies. In dichloroacetyl chloride group, anti-cardiolipin antibodies. No difference in anti-histone, -Sm, or -DNA antibodies	Not evaluated	Not evaluated	Khan et al. (1995)
6 per group, 0 (corn oil), 0.2 mmol/kg dichloroacetyl chloride, or 0.2 mmol/kg dichloroacetic anhydride, 2 times per wk for 6 wks	LOAEL: 0.2 mmol/kg TCE, 0.2 mmol/kg dichloroacetic anhydride	In both treated groups, increased ANA	In both treated groups, increased IL-1α, IL-1ß, IL-3, IL-6, IFN-γ, G-CSF and keratinocyte-derived chemokine (KC) secretion; decreased IL-5. In dichloroacetyl chloride group, increased IL-17 and INF-α[d]	In both treated groups, increased lymphocytes in spleen, thickening of alveolar septa with lymphocytic interstitial infiltration	Cai et al. (2006)
Autoimmune-prone: Female NZB × NZW Mice, Drinking Water					
6 per group, 0, 1400, or 14,000 ppb TCE[e,f], 27 wks exposure	LOAEL: 1,400 ppb	Increased anti-dsDNA antibodies at 19 wks and at 32-32 wks in the 1,400 ppb group	Not evaluated	At 14,000 ppb, proteinuria increased beginning at 20 wks; renal pathology scores increased, no evidence of liver disease	Gilkeson et al. (2004)
10 per group, 0, 1400, or 14,000 ppb TCE[f], 27 wks exposure	LOAEL: 1,400 ppb	Increased anti-dsDNA antibodies at 19 wks and at 32-32 wks in the 1,400 ppb group	No effect on splenocyte NK activity	No effect on renal pathology score; liver disease not examined	Kiel et al. (2009)

Table 4-67. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	*Ex vivo* assays of cultured splenocytes	Clinical and histopathology	
Autoimmune-prone: Male MRL—*lpr/lpr* Mice, Inhalation					
5 per group, 0, 500, 1000, or 2,000 ppm TCE, 4 h/d, 6 d/wk, 8 wks	LOAEL: 500 ppm			At ≥500 ppm, dose-related liver inflammation, splenomegaly and hyperplasia of lymphatic follicles; at 1,000 ppm, immunoblastic cell formation in lymphatic follicles, no changes in thymus	Kaneko et al. (2000)
Autoimmune-inducible: Female Brown Norway Rat, Gavage					
6-8 per group, 0, 100, 200, 400 mg/kg, 5 d/wk, 6 wks followed by 1 mg/kg $HgCl_2$ challenge	NOAEL 500 mg/kg	Not reported[g]	Not evaluated	Not evaluated	White et al. (2000)
Nonautoimmune-prone: Female B6C3F1 Mice, Drinking Water					
6 per group, 0, 1400, or 14,000 ppb TCE,[e,f] 30 wks exposure	LOAEL: 1,400 ppb	Anti-dsDNA increased in 1,400 ppb group beginning at age 32 wks and in the 14,000 ppb group beginning at age 26 wks	No effect on splenocyte NK activity	No renal disease observed	Gilkeson et al. (2004)

Table 4-67. Summary of autoimmune-related studies of TCE and metabolites (by sex, strain, and route of exposure) (continued)

Number/group, vehicle, dose, duration	NOAEL; LOAEL[b]	Results			Reference
		Serology	*Ex vivo* assays of cultured splenocytes	Clinical and histopathology	
10 per group, 0, 1400, or 14,000 ppb TCE,[f] 30 wks exposure	LOAEL: 1,400 ppb	Anti-dsDNA increased beginning at 26 wks in the 14,000 ppb group and at 32 wks of age in the 1,400 ppb group; increases in anti-ssDNA antibodies seen in both groups at 32 wks. Anti-GA were not affected	No effect on splenocyte NK activity	Increased renal pathology scores in 1,400 ppb group; Significant decrease in thymus weight in both groups	Kiel et al. (2009)

[a]Selected endpoints, based on those reported across the majority of studies. Lupus-prone mouse strains develop lupus-like condition spontaneously, with virtually complete penetrance. The autoimmune-inducible (Brown Norway) rat has been used as a model of mercuric chloride induced glomerulonephritis and experimental autoimmune myasthenia gravis.

[b]NOAEL and LOAEL are based upon reported study findings.

[c]No difference reported in anti- ds-DNA, -ss-DNA, -ribonucleosome, -SSA, -SSB, -Sm, -Jo-1, or -Scl-70 antibodies.

[d]No difference reported in secretion of other cytokines measured: IL-2, IL-4, IL-10, IL-12, TNF-α, granulocyte monocyte colony stimulating factor, macrophage inflammatory protein-1α, and RANTES (CCL-5).

[e]Dose levels cited in the report (Gilkeson et al., 2004) were incorrect; corrections provided by personal communication from Margie Peden-Adams (Medical University of South Carolina) to Glinda Cooper (U.S. EPA) on 13 August 2008; dose levels in this table are correctly report.

[f]Dose in mg/kg/d not given.

[g]Anti-dsDNA tests were described in the methods section; no effect of TCE on serum IgE levels was seen, and it is not clear if the additional serological tests were conducted in the TCE portion of this study or if they were conducted but not reported because no effect was seen.

In a subsequent study which assessed occupationally relevant concentrations, TCE was administered to female MRL +/+ mice (8/group) in drinking water at treatment levels of 0.1, 0.5, or 2.5 mg/mL (21, 100, or 400 mg/kg/d) for 4 and 32 weeks (Griffin et al., 2000b). At 4 weeks, significant increases in serum antinuclear antibody levels were observed at 0.1 and 0.5 mg/kg/d;at 32 weeks, the effects were observed at all three treatment levels. A dose-related increase in the percentage of activated CD4+ T-cells in spleens and lymph nodes of treated mice was observed at 32 weeks, and the CD4+ T-cells were found to secrete Th1-type cytokines at 4 and 32 weeks.

A similar response was observed by Cai et al. following chronic (48 weeks) exposure of TCE to female MRL +/+ mice (5/group) in drinking water at 0 or 0.5 mg/mL (approximately 60 μg/g/day) (Cai et al., 2008). After 11 weeks of treatment, a statistically significant decrease in body weight gain was observed. After 24 weeks of exposure, serum ANA were consistently elevated in treated mice as compared to control, although statistical significance was not achieved. Apparent treatment-related effects on serum cytokines included decreased IL-6 after 36 and 48 weeks, decreased TNF-α after 48 weeks, and increased granulocyte colony stimulating factor (G-CSF) after 36 weeks of treatment. After 36 weeks of treatment, *ex vivo* cultured splenocytes secreted higher levels of IFN-γ than control splenocytes. Although there were no observed effects on serum aminotransferase liver enzymes at termination, statistically significant incidences of hepatocytic necrosis and leukocyte infiltration (including CD3+ T lymphocytes) into liver lobules were observed in treated mice after 48 weeks of exposure. Hepatocyte proliferation was also increased. TCE treatment for 48 weeks also induced necrosis and extensive infiltration of leukocytes in the pancreas, infiltration of leukocytes into the perivascular and peribronchial regions of the lungs, and thickening of the alveolar septa in the lungs. At 36 and 48 weeks of exposure, massive perivascular infiltration of leukocytes (including CD3+ T lymphocytes) was observed in the kidneys, and immunoglobulin deposits were found in the glomeruli.

To examine the role of metabolic activation in the autoimmune response, Griffin et al. (2000c) treated MRL +/+ mice with 2.5 mg/mL (300 mg/kg/d) TCE in drinking water for 4 weeks (Griffin et al., 2000c). Immune responses were examined in the presence or absence of subcutaneous doses of 200 mg/kg/d diallyl sulfide, a specific inhibitor of CYP2E1 which is known to be a primary CYP cytochrome that is active in TCE metabolism. With diallyl sulfide cotreatment that resulted in a decreased level of CYP2E1 apoprotein in liver microsomes, the enhanced mitogen-induced proliferative capacity of T-cells was inhibited and the reduction in IL-4 levels secreted by CD4+ T-cells was reversed for TCE-treated MRL +/+ mice. This study

suggests that metabolism of TCE by CYP2E1 is responsible, at least in part, for the treatment-related CD4+ T-cell alterations.

The TCE metabolite, trichloroacetaldehyde (TCAA) or trichloroacetaldehyde hydrate (TCAH), was also evaluated in MRL +/+ mice (Blossom et al., 2007; Blossom and Gilbert, 2006; Gilbert et al., 2004) in order to determine if outcomes similar to the immunoregulatory effects of TCE would be observed, and to attempt to further characterize the role of metabolism in the mode of action for TCE. At concentrations ranging from 0.04 to 1 mM, TCAA stimulated proliferation of murine Th1 cells treated with anti-CD3 antibody or antigen *in vitro*. At similar concentrations, TCAA induced phenotypic alterations consistent with upregulation of CD28 and downregulation of CD62L in cloned memory Th1 cells and DC4+ T-cells from untreated MRL +/+ mice. Phosphorylation of activating transcription factor 2 (ATF-2) and c-Jun (two components of the activator protein-a transcription factor) was also observed with TCAA-induced Th1 cell activation. Higher concentrations of TCAA formed a Schiff base on T-cells, which suppressed the ability of TCAA to phosphorylate ATF-2. These findings suggested that TCAA may promote T-cell activation by stimulating the mitogen-activated protein kinase pathway in association with Schiff base formation on T-cell surface proteins (Gilbert et al., 2004).

In order to determine whether metabolites of TCE could mediate the immunoregulatory effects previously observed with TCE treatment (i.e., the generation of lupus and autoimmune hepatitis, associated with activation of IFN-γ-producing CD4+ T-cells), Blossom et al. (2004) administered TCE metabolites, TCAH and trichloroacetic acid (TCA), to MRL +/+ mice (6−8/group) in drinking water for 4 weeks. Drinking water concentrations were 0, 0.1, or 0.9 mg/mL; average daily doses were calculated as 0, 24, or 220 mg/kg/d for TCAH and 0, 27, or 205 mg/kg/d for TCA. These treatment levels were considered to be physiologically relevant and to reflect occupational exposure. A phenotypic analysis of splenic and lymph node cells, cytokine profile analysis, evaluation of apoptosis in CD4+ T-cells, and examination of serum markers of autoimmunity (anti-ssDNA, anti-histone, or ANA) were conducted. Exposure to TCAH or TCA at both treatment levels was found to promote CD4+ T-cell activation, as shown by significant ($p < 0.05$) increases in the percentage of $CD62L^{lo}$ CD4+ T-cells in the spleens and lymph nodes of the MRL +/+ mice. Increased levels of IFN-γ were secreted by CD4+ T-cells from mice treated by TCAH and TCA. No significant changes in body weight were observed; spleen weights were similar between control and treated mice with the exception of a significant decrease in spleen weight from mice treated with 0.9 mg/mL TCA. Liver and kidney histology were not affected, and serum alanine aminotransferase levels were similar for control and treated mice. A generalized trend towards an increase in serum autoantibodies (anti-ssDNA) was

observed in TCAH-treated mice, and slight but significant increases in anti-histone and anti-nuclear antibody production were observed in mice treated with 0.9 mg/mL-day TCAH.

The autoimmune response of female MRL +/+ mice to DCAC, a metabolite of TCE, and to dichloroacetic anhydride (DCAA) a similar acylating agent, was evaluated by Cai et al. (2006). Six mice/group were injected intraperitoneally, twice weekly for 6 weeks, with 0.2 mmol/kg DCAC or DCAA in corn oil. Body weight gain was significantly decreased after 5 or 6 weeks treatment with DCAC and DCAA. DCAC treatment resulted in significant increases in total serum IgG (77% increase over control) and IgG1 (172% increase over control), as well as the induction of DCAC-specific IgG and IgG1. Serum IgM levels were significantly decreased by 25 and 18% in DCAC and DCAA-treated mice, respectively. IgE levels were increased 100% over controls in DCAC-treated mice. Of eight Th1/Th2 cytokines measured, only IL-5 was decreased in DCAC- and DCAA-treated mice. Serum ANA were detected in both DCAC- and DCAA-treated mice. Treatment-related increases in cytokine and chemokine secretion in cultured splenocytes were observed for DCAC and DCAA (IL-1, G-CSF, KC, IL-3, and IL-6). DCAC-treated splenocytes also secreted more IL-17 and IFN-α than controls. Histopathological changes were observed in the spleens of DCAC and DCAA-treated mice (lymphocyte population increases in the red pulp). With both DCAC and DCAA treatment, the alveolar septa were thickened in the lungs, moderate levels of lymphocytic interstitial infiltrates were present in tissues, and alveolar capillaries were clogged with erythrocytes. These findings were attributed both to the predisposition of the MRL +/+ mice towards autoimmune disease, and to the treatment-related induction of autoimmune responses.

Fas-dependant activation-induced cell death leading to autoimmune disease has been shown to be related to impaired Fas or FasL ligand expression in humans and mice, and defects in the Fas-signaling pathways have been described in autoimmune disease models. The study by Blossom and Gilbert examined the effects of TCAH on Fas-dependent autoimmune cell death (Blossom and Gilbert, 2006). In this study, TCAH (1) inhibited apoptosis of antigen-activated cells, (2) did not protect CD4+ T-cells from Fas-independent apoptosis, (3) did not inhibit autoimmune cell death induced by direct engagement of the Fas receptor, (4) inhibited the expression of FasL but not Fas on the surface of activated CD4+ T-cell, (5) increased release of FasL from CD4+ cells in a metalloprotein-dependent manner, and (6) increased metalloprotein MMP-7 expression.

Gilbert et al. (2006) studied the effect of treatment on apoptosis in CD4+ T-lymphocytes isolated from MRL +/+ female mice that had been exposed to TCE (0, 0.1, 0.5, or 2.5 mg/mL) in the drinking water for 4 or 32 weeks or to TCAH (0.1, 0.3, or 0.9 mg/mL) in drinking water for 4 or 40 weeks. After only 4 weeks, decreased activation-induced apoptosis was associated with

decreased FasL expression in the CD4+ T-cells, suggesting that TCE- and TCAH-induced autoimmune disease was promoted through suppression of the process that would otherwise delete activated self-reactive T-lymphocytes. By 32 weeks of treatment, TCE had induced autoimmune hepatitis, which was associated with the promotion of oxidative stress, the formation of liver protein adducts, and the stimulated production of antibodies to those adducts. TCAH-treated mice did not exhibit autoimmune hepatitis by 40 weeks, but developed a dose-dependant alopecia and skin inflammation (Blossom et al., 2007). TCAH appeared to modulate the CD4+ T-cell subset by promoting the expression of an activated/effector phenotype with an increased capacity to secrete the proinflammatory cytokine IFN-γ. A 4-week exposure to TCAH attenuated activation-induced cell death and the expression of the death receptor Fas in CD4+ cells; these effects were not seen after a 40-week exposure period. Differences in response were tentatively attributed to higher levels of metalloproteinases (specifically MMP-7) at 4-weeks of treatment, suggesting a possible mechanism for the promotion of skin pathology by TCAH.

The role of protein adduct formation in autoimmune response has been pursued by various researchers. Halmes et al. administered a single i.p. dose of TCE in corn oil to male Sprague-Dawley rats (2/group) at 0 or 1,000 mg/kg (Halmes et al., 1997). Using antiserum that recognizes TCE covalently bound to protein, a single 50 kDa microsomal adduct was detected by Western blot in livers of treated rats. Using affinity chromatography, a 50 kDa dichloroacetyl protein was also isolated from rat plasma. The protein was reactive immunochemically with anti-CYP2E1 antibodies. The data suggest that the protein adduct may be CYP2E1 that has been released from TCE-damaged hepatocytes.

Cai et al. examined the role of protein haptenization in the induction of immune responses (Cai et al., 2007). In this study, MRL +/+ mice were immunized with albumin adducts of various TCE reactive intermediates of oxidative metabolism. Serum immunoglobulins and cytokine levels were measured to evaluate immune responses against the haptenized albumin. Antigen-specific IgG responses (subtypes: IgG1, IgG2a, and IgG2b) were found. Serum levels of G-CSF were increased in immunized mice, suggesting macrophage activation. Following immunization with formyl-albumin, lymphocyte infiltration in the hepatic lobule and portal area was increased. This study suggests that proteins that are haptenized by metabolites of TCE may act as antigens to induce humoral immune responses and T-cell-mediated hepatitis.

A possible role for oxidative stress in inflammatory autoimmune disease was proposed by Khan et al. (2001). A study was performed in which female MRL +/+ mice were treated with 10 mmol/kg TCE or 0.2 mmol/kg DCAC via intraperitoneal injection every 4th day for 2, 4, 6, or 8 weeks. Anti-malondialdehyde serum antibodies, a marker of lipid peroxidation and oxidative stress, were measured and were found to increase by 4 weeks of treatment, marginally for TCE

and significantly for DCAC. It was reported that anti-malondialdehyde antibodies has also been found to be present in the serum of systemic lupus erythematosus-prone MRL-lpr/lpr mice.

In another study that addressed the association of oxidative and nitrosative stress, and the role of lipid peroxidation and protein nitration, in TCE-mediated autoimmune response, Wang et al. treated female MRL +/+ mice with 0.5 mg/mL TCE in drinking water for 48 weeks (Wang et al., 2007b). The formation of antibodies in the serum to lipid peroxidation-derived aldehyde protein adducts was evaluated. With TCE treatment, the serum levels of anti-malondialdehyde and anti-4-hydroxynonenal protein adduct antibodies, inducible nitric oxide synthase, and nitrotyrosine were increased. These were associated with increases in anti-nuclear-, anti-ssDNA- and anti-dsDNA antibodies. The involvement of lipid peroxidation-derived aldehyde protein adducts in TCE autoimmunity was further explored, using female MRL +/+ mice that were administered by i.p. injections of TCE at 10 mmol/kg, either every 4^{th} day for 6 or 12 weeks (Wang et al., 2007a) or once per week for 4 weeks (Wang et al., 2008). Significant increases in malondialdehyde and 4-hydroxynonenal protein adducts, as well as significant induction of specific antibodies directed against these antigens were observed in both studies. Wang et al. also demonstrated a significant proliferation of CD4+ T-cells in TCE-treated mice, and splenic lymphocytes from TCE-treated mice released more IL-2 and IFN-γ when stimulated with MDA- or 4-hydroxynonenal-adducted mouse serum albumin (Wang et al., 2008). Overall, the result of these studies suggest a role for lipid peroxidation aldehydes in the induction and/or exacerbation of autoimmune response in the MRL +/+ animal model, and the involvement of Th1 cell activation.

In studies conducted in other rodent strains, less consistent outcomes have been observed. Inhalation exposure of an autoimmune-prone strain of male mice (MRL-lpr/lpr) to 0-, 500-, 1,000-, or 2,000-ppm TCE for 4 hours/day, 6 days/week, for 8 weeks resulted in depressed serum IgG levels and increased numbers of lymphoblastoid cells (Kaneko et al., 2000). Also at 2,000 ppm, changes in T-cell helper to suppressor cell ratios were observed. At histopathological evaluation, dose-dependent inflammation and associated changes were noted in the liver at ≥500 ppm, hyperplasia of the lymphatic follicles of the spleen and splenomegaly were observed at ≥500 ppm, and the spleen exhibited the development of an immunoblastic-cell-like structure at 1,000 ppm.

A 26-week drinking water study of TCE in NZB × NZW (NZBWF1) autoimmune-prone mice demonstrated an increase in anti-dsDNA antibodies at 19 weeks and at 32 and 34 weeks in the 1,400 ppb group, and increased kidney disease at 14,000 ppb (i.e., increased proteinuria at 20 weeks; increased renal pathology scores at termination, based upon glomerular proliferation,

inflammation, and necrosis) (Gilkeson et al., 2004).[1] Also in that study, a small increase in anti-dsDNA antibody production, without kidney disease, was observed in B6C3F1 mice, with statistically significant ($p < 0.05$) or borderline ($p = 0.07$) effects seen in the 1,400-ppb group at observations between 32 and 39 weeks of age, and in the 14,000 ppb group at observations between 26 and 39 weeks of age.

Keil et al. (2009) also assessed the effects of TCE exposure on NZWBF1 mice, comparing the responses to those of TCE-exposed B6C3F1 mice, which are not autoimmune prone (Keil et al. 2009). In this study, groups of NZWBF1 and B6C3F1 female mice (10/dose level) were administered 0, 1400, or 14,000 ppb TCE in the drinking water. Treatment was initiated at 9 weeks of age and continued until 36 weeks of age for the NZBWF1 and until 39 weeks of age for the B6C3F1 mice. Body weight; spleen, thymus, liver, and kidney weight; spleen and thymus cellularity; and renal pathology were assessed. Splenic lymphocyte proliferation, autoantiboidy production (anti-dsDNA, anti-ssDNA, and anti-glomerular), total serum IgG, NK cell activity, and mitogen-induced lymphocyte proliferation were conducted. Administration of TCE did not result in alterations in NK cell activity or T- or B-cell proliferation in either strain of mice. In the NZBWF1 mice, there was little evidence of an increase or of an acceleration in ss-DNA antibody production with TCE exposure, but as was seen in the earlier study by these investigators (Gilkeson et al., 2004), ds-DNA antibodies were increased at 19 weeks and at 32−34 weeks in the 1,400 ppb group. However, anti-glomerular antibody levels were increased in NZBWF1 mice early in the study, returning to control levels by 23 weeks of age. In the B6C3F1 mice the number of activated T-cells (CD4++/CD44+) was increased (significantly at 14,000 ppm; $p \leq 0.05$) and thymus weights were significantly decreased ($p \leq 0.05$) in a dose-responsive manner. Renal pathology (as indicated by renal score based on assessment of glomerular inflammation, proliferation, crescent formation and necrosis) was significantly increased ($p \leq 0.05$) at 1,400 ppm. Also in the B6C3F1 mice, autoantibodies to dsDNA were increased relative to controls beginning at 26 weeks in the 14,000-ppb group and at 32-weeks of age in the 1,400 ppb group; increases in anti-ssDNA antibodies were seen in both groups at 32 weeks. Anti-glomerular antibodies were not affected in B6C3F1 mice. In summary, the authors concluded that this study showed that 27−30 weeks of TCE drinking water administration to NZBWF1 (autoimmune-prone) mice did not contribute to the progression of autoimmune disease, while similar administration to B6C3F1 (nonautoimmune-prone) mice increased the expression of a number of markers that are associated with autoimmune disease.

[1]The study was reported in symposium proceedings. Dose levels cited in the proceedings were incorrect; however, corrections were provided by personal communication from Margie Peden-Adams (Medical University of South Carolina) to Glinda Cooper (U.S. EPA) on 13 August 2008, and dose levels are correctly reported here.

This study is important in that it demonstrates that autoimmune responses to TCE exposure in animal models are not solely dependant upon a genetic predisposition to autoimmune disease.

White et al. conducted a study in female Brown Norway rats, which have been shown to be susceptible to development of chemically-induced IgE mediated glomerulonephritis that is similar to the nephritic damage seen in systemic lupus erythematosus (White et al., 2000). TCE administered by gavage 5 days/week at 100, 200, or 400 mg/kg did not increase in IgE levels after 6 weeks exposure, or after an additional challenge with 1 mg/kg mercuric chloride ($HgCl_2$).

Several studies have examined the potential for autoimmune response following oral exposures during pre- and postnatal immune system development, as described in Section 4.6.2.1.2 above. Peden-Adams et al. conducted two such studies. In the first study, B6C3F1 mice were treated with either 1,400 or 14,000 ppb TCE in drinking water from gestation Day 0 to postnatal Week 8 (Peden-Adams et al., 2006). No treatment-related increases in serum anti-ds-DNA antibody levels were observed in the 8-week old offspring, although it is noted that the mouse strain used in the experiment is not an autoimmune-prone animal model. A more recent study (Peden-Adams et al., 2008) exposed pregnant MRL +/+ mice to TCE in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continued the exposures until the offspring were 12 months of age. Consistent with the findings of the 2006 publication, autoantibody levels (anti-dsDNA and anti-glomerular) were not increased in the offspring over the course of the study. Contrasting with these negative studies, the lupus-prone MRL +/+ mouse model was utilized in two additional drinking water studies with developmental exposures in which there was some indication of a positive association between developmental exposures to TCE and the initiation of autoimmune disease. Blossom and Doss (2007) administered TCE to pregnant MRL +/+ mice in drinking water at levels of 0, 0.5, or 2.5 mg/mL and continued administration to the offspring until approximately 7−8 weeks of age. TCE exposure induced a dose-dependent increase in T-lymphocyte IFN-γ in peripheral blood at 4−5 weeks of age, but this effect was not observed in splenic T-lymphocytes at 7−8 weeks of age. Serum anti-histone autoantibodies and total IgG_{2a} were significantly increased in the TCE-treated offspring; however, histopathological evaluation of the liver and kidneys did not reveal any treatment-related signs of autoimmunity. In a study by Blossom et al. (2008), pregnant MRL +/+ mice were administered TCE in the drinking water at levels of 0 or 0.1 mg/mL from GD 0 through lactation, and continuing postweaning in the offspring until postnatal Day 42. Significant treatment-related increases in pro-inflammatory cytokines (IFN-γ and Il-2 in males and TNF-α in both sexes) produced by splenic CD4+ T-cells were observed in PND 42 offspring.

In summary, TCE treatment induces and exacerbates autoimmune disease in genetically susceptible strains of mice, and has also been shown to induce signs of autoimmune disease in a

nongenetically predisposed strain. Although the mechanism for this response is not fully understood, a number of studies have been conducted to examine this issue. The primary conclusion to date is that metabolism of the TCE to its chloral or dichloroacetic acid metabolites is at least partially responsible for activating T-cells or altering T-cell regulation and survival associated with polyclonal disease in susceptible mice strains.

4.6.2.4. *Cancers of the Immune System*

Cancers of the immune system that have been observed in animal studies and are associated with TCE exposure are summarized in Tables 4-68 and 4-69. The specific tumor types observed are malignant lymphomas, lymphosarcomas, and reticulum cell sarcomas in mice and leukemias in rats.

In the NCI (1976) study, the results for Osborne-Mendel rats were considered inconclusive due to significant early mortality, but exposure to B6C3F1 mice were also analyzed. Limited increases in lymphomas over controls were observed in both sexes of mice exposed (see Table 4-68). The NCI study (1976) used technical grade TCE which contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane). A later study (Henschler et al., 1984) in which mice were given TCE that was pure, industrial, and stabilized with one or both of these stabilizers did not find significant increases in lymphomas over historical controls. A gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, did not see an increase in lymphomas in all four strains of rats (ACI, August, Marshall, and Osborne-Mendel). The final NTP study (1990) in male and female F344 rats and B6C3F1 mice, using epichlorohydrin-free TCE, again experienced early mortality in male rats. This study did not observe significant increase in lymphomas over that of controls. Henschler et al. (1980) tested NMRI mice, WIST rats and Syrian hamsters of both sexes, and observed a variety of tumors in both sexes (Henschler et al., 1980), consistent with the spontaneous tumor incidence in this strain (Deerberg and Muller-Peddinghaus, 1970; Deerberg et al., 1974). Henschler et al. did not show an increase in lymphomas in rats or hamsters of either sex (Henschler et al., 1980). Background levels of lymphomas in this mouse strain are high, making it difficult to determine if the increased lymphomas in female mice is a treatment effect. In a follow-up study, Henschler et al. (1984) examined the role of stabilizers of TCE in the lymphomas demonstrated in female mice in the 1980 paper. Each exposure group had ~50 SPF-bred ICR/HA-Swiss mice and exposure was for 18 months. Background incidence of tumors was high in all groups. Focusing just on malignant lymphomas (see Table 4-68), the high background incidence in unexposed animals again makes it difficult to determine if there is TCE and/or stabilizer-related incidence of lymphomas. There are no data at any other timepoint than

18 months. A high mortality rate in all animals as well as the increased incidence of 'background' lymphomas in that report was also a problem and may have been related to the shorter time frame.

Table 4-68. Malignant lymphomas incidence in mice exposed to TCE in gavage and inhalation exposure studies

Cancer type, species, and sex	Exposure groups						Reference
Gavage exposure							
Malignant lymphomas							NTP, 1990[a]
Prevalence in: (*n* affected/total)	Vehicle control		1,000 mg/kg/d				
B6C3F1 mice, male	11/50 (22%)		13/50 (26%)				
B6C3F1 mice, female	7/48 (15%)		13/49 (27%)				
Lymphosarcomas and reticulum cell sarcomas							NCI, 1976[b]
Prevalence in: (*n* affected/total)	Vehicle control		Low dose		High dose		
B6C3F1 mice, male	1/20 (5%)		4/50 (8%)		2/48 (4%)		
B6C3F1 mice, female	1/20 (5%)		5/50 (10%)		5/47 (11%)		
Malignant lymphomas							Henschler et al., 1984[c]
Prevalence in: (*n* affected/total)	Control	TCE-pure	TCE-indust	TCE-EPC	TCE-BO	TCE-EPC-BO	
Swiss (ICR/HA) mice, male	19/50 (38%)	16/50 (32%)	17/49 (35%)	11/49 (22%)	11/49 (22%)	12/49 (24%)	
Swiss (ICR/HA) mice, female	28/50 (56%)	21/50 (42%)	19/50 (38%)	20/50 (40%)	23/48 (48%)	18/50 (36%)	
Inhalation exposure							
Malignant lymphomas	Control		96		480		Henschler et al., 1980[d]
Prevalence in: (*n* affected/total)							
Han:NMRI mice, male	7/30 (23%)		7/29 (24%)		6/30 (20%)		
Han:NMRI mice, female[e]	9/29 (31%)		17/30 (57%)		18/28 (64%)		

[a]After 103 weeks gavage exposure, beginning at 8 weeks of age.
[b]After 90 weeks gavage exposure, beginning at 5 weeks of age. Low dose is 1,200 mg/kg/d for male mice, 900 mg/kg/d for female mice (5 days/week). High dose is 2,400 mg/kg/d for male mice, 1,800 mg/kg/d for female mice (5 days/week).
[c]After 72 weeks gavage exposure (corn oil), beginning at 5 weeks of age. Male mice received 2,400 mg/kg/d, female mice received 1,800 mg/kg/d. Stabilizers were added in the percent w/w: TCE-EPC, 0.8%, TCE-BO, 0.8%, TCE-EPC-BO, 0.25 and 0.25%.
[d]After 78 weeks inhalation exposure. Administered daily concentration: low dose is 96 (mg/m^3) and high dose is 480 (mg/m^3), equivalent to 100 and 500 ppm (100 ppm = 540 mg/m^3), adjusted for 6 hours/day, 5 days/week exposure.
[e]Statistically significant by Cochran-Armitage trend test ($p < 0.05$).

Sources: NTP (1990) Tables 8, 9; NCI (1976) Table XXXa; Henschler et al. (1980) Table 3a.

Table 4-69. Leukemia incidence in rats exposed to TCE in gavage and inhalation exposure studies

Species and sex	Exposure groups				Reference
Gavage exposure					
Prevalence in (*n* affected/total)	Control	50 mg/kg	250 mg/kg		Maltoni et al., 1986 [a]
Sprague-Dawley rats, male	0/30 (0%)	2/30 (6.7%)	3/30 (10.0%)		
Sprague-Dawley rats, female	1/30 (3.3%)	0/30 (0%)	0/30 (0%)		
	Control	500 mg/kg	1,000 mg/kg		NTP, 1988 [b]
August rats, female	0/50 (0%)	1/50 (2%)	5/50 (10%)		
Inhalation exposure					
Prevalence in (*n* affected/total)	Control	100 ppm	300 ppm	600 ppm	Maltoni et al., 1988 [c]
Sprague-Dawley rats, male	9/135 (6.7)	13/130 (10.0)	14/130 (10.8)	15/130 (11.5)	
Sprague-Dawley rats, female	7/145 (4.8)	9/130 (6.9)	2/130 (1.5)	11/130 (8.5)	

[a]After 52 weeks gavage exposure, beginning at 13 weeks of age, olive oil vehicle. Percent affected and starting *n* given in reported; U.S. EPA calculated *n* affected.
[b]After 104 weeks gavage exposure, beginning at 6.5−8 weeks of age, corn oil vehicle.
[c]After 104 weeks inhalation exposure, BT304 and BT304bis. Percent affected and starting *n* given in reported; U.S. EPA calculated *n* affected.

Maltoni et al reported a nonsignificant increase in leukemias in male rats exposed via inhalation (Matoni et al., 1988, 1986). Maltoni et al. (1986) demonstrates a borderline higher frequency of leukemias in male Sprague-Dawley rats following exposure by ingestion for 52 weeks, believed by the authors to be related to an increase in lymphoblastic lymphosarcomas (see Table 4-69). The gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, observed leukemia in female August rats with a positive trend, but was not significantly greater than the vehicle controls.

In summary, overall there is limited available data in animals on the role of TCE in lymphomas and leukemias. There are few studies that analyze for lymphomas and/or leukemias. Lymphomas were described in four studies (NTP, 1990; NCI, 1976; Henschler et al., 1980, 1984) but study limitations (high background rate) in most studies make it difficult to determine if these are TCE-induced. Three studies found positive trends in leukemia in specific strains and/or gender (Maltoni et al., 1986, 1988; NTP, 1988). Due to study limitations, these trends can not be determined to be TCE-induced.

4.6.3. Summary

4.6.3.1. *Noncancer Effects*

The human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome. The data pertaining to immunosuppressive effects is weaker.

The relation between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies. A meta-analysis of scleroderma studies (Diot et al., 2002; Garabrant et al., 2003; Nietert et al., 1998) conducted by the U.S. EPA resulted in a statistically significant combined odds ratio for any exposure in men (OR: 2.5, 95% CI: 1.1, 5.4), with a lower relative risk seen in women in women (OR: 1.2, 95% CI: 0.58, 2.6). The incidence of systemic sclerosis among men is very low (approximately 1 per 100,000 per year), and is approximately 10 times lower than the rate seen in women (Cooper and Stroehla, 2003). Thus, the human data at this time do not allow us to determine if the difference in effect estimates between men and women reflects the relatively low background risk of scleroderma in men, gender-related differences in exposure prevalence or in the reliability of exposure assessment (Messing et al., 2003), a gender-related difference in susceptibility to the effects of TCE, or chance. Changes in levels of inflammatory cytokines were reported in an occupational study of degreasers exposed to TCE (Iavicoli et al., 2005) and a study of infants exposed to TCE via indoor air (Lehmann et al., 2001, 2002). Experimental studies support the biological plausibility of these effects. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice (Cai et al., 2008; Blossom et al., 2007, 2004; Griffin et al., 2000a, b). With shorter exposure periods, effects include changes in cytokine levels similar to those reported in human studies. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and interestingly, these effects differ somewhat from the "normal" expression in these mice. Immunotoxic effects, including increases in anti-ds DNA antibodies in adult animals and decreased plaque forming cell response with prenatal and neonatal exposure, have been also reported in B6C3F1 mice, which do not have a known particular susceptibility to autoimmune disease (Gilkeson et al., 2004, Peden-Adams et al., 2006). Recent mechanistic studies have focused on the roles of various measures of oxidative stress in the induction of these effects by TCE (Wang et al., 2008, 2007b).

There have been a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to TCE, with prevalences as high as 13% of workers in the same location (Kamijima et al., 2008, 2007). Evidence of a treatment-related increase in delayed hypersensitivity response

accompanied by hepatic damage has been observed in guinea pigs following intradermal injection (Tang et al., 2008, 2006), and hypersensitivity response was also seen in mice exposed via drinking water pre- and postnatally (gestation Day 0 through to 8 weeks of age) (Peden-Adams et al., 2006).

Human data pertaining to TCE-related immunosuppression resulting in an increased risk of infectious diseases is limited to the report of an association between reported history of bacteria of viral infections in Woburn, Massachusetts (Lagakos, 1986). Evidence of localized immunosuppression, as measured by pulmonary response to bacterial challenge (i.e., risk of Streptococcal pneumonia-related mortality and clearance of Klebsiella bacteria) was seen in an acute exposure study in CD-1 mice (Aranyi et al., 1986). A 4-week inhalation exposure in Sprague-Dawley rats reported a decrease in plaque forming cell response at exposures of 1,000 ppm (Woolhiser et al., 2006).

4.6.3.2. *Cancer*

Associations observed in epidemiologic studies of lymphoma and TCE exposure suggest a causal relation between trichloroethylene exposure and lymphoma. Issues of study heterogeneity, potential publication bias, and weaker exposure-response results contribute uncertainty to the evaluation of the available data.

In a review of the lymphoma studies, 17 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 on TCE exposure patterns and factors that affect such exposure) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified. These studies generally reported excess relative risk estimates for lymphoma between 0.8 and 3.1 for overall TCE exposure. Statistically significant elevated relative risk estimates with lymphoma and overall TCE exposure were observed in two cohort (Hansen et al., 2001; Raaschou-Nielsen et al., 2003) and one case-control (Hardell et al., 1994) study. Both cohort studies reported statistically significant associations with lymphoma for subjects with longer employment duration as a surrogate of TCE exposure. Hardell et al. (1994) reported a strong but imprecise association, in part reflecting possible bias from subject-reported exposure history and few exposed cases. Other high-quality studies reported a 10 to 50% elevated relative risk estimate with overall TCE exposure that were not statistically significant, except for two population case-control studies of lymphoma, which did not report relative risk estimates with overall TCE exposure but did for medium-high intensity or cumulative TCE exposure (Miligi et al., 2006; Seidler et al., 2007). Fifteen additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that

would decrease study power and sensitivity. The observed lack of association with lymphoma in these studies likely reflects study design and exposure assessment limitations and is not considered inconsistent with the overall evidence on TCE and lymphoma.

Consistency of the association between TCE exposure and lymphoma is further supported by the results of meta-analyses of 16 high-quality studies reporting risk estimates for overall TCE exposure. These meta-analyses found a statistically significant increased pooled relative risk estimate for lymphoma of 1.23 (95% CI: 1.04, 1.44) for overall TCE exposure. The analysis of lymphoma was robust to the removal of individual studies and the use of alternate relative risk estimates from individual studies, and in only one cases was the resulting pooled relative risk no longer statistically significant (lower 95% confidence bounds of 1.00). Some evidence heterogeneity was observed, particularly between cohort and case-control studies, but it was not statistically significant ($p = 0.10$); and, in addition, there was some evidence of potential publication bias. Analyzing the cohort and case-control studies separately resolved most of the heterogeneity, but the result for the pooled case-control studies was only a 7% increased relative risk estimate and was not statistically significant. The sources of heterogeneity are uncertain but may be the result of some bias associated with exposure assessment and/or disease classification, or from differences between cohort and case-control studies in average TCE exposure.

Exposure-response relationships are examined in the TCE epidemiologic studies only to a limited extent. Many studies examined only overall "exposed" versus "unexposed" groups and did not provide exposure information by level of exposure. Others do not have adequate exposure assessments to confidently distinguish between levels of exposure. The lymphoma case-control study of Seidler et al. (2007) reported a statistically significant trend with TCE exposure ($p = 0.03$ for Diffuse B-cell lymphoma trend with cumulative TCE exposure), and lymphoma risk in Boice et al. (1999) appeared to increase with increasing exposure duration ($p = 0.20$ for routine-intermittent exposed subjects). The borderline statistically significant trend with TCE intensity in the case-control study of Wang et al. (2009 [$p = 0.06$]) is consistent with Seidler et al. (2007). Further support was provided by meta-analyses using only the highest exposure groups, which yielded a higher pooled relative risk estimate (1.57 [95% CI: 1.27, 1.94]) than for overall TCE exposure (1.27 [95% CI: 1.04, 1.44]).

Few risk factors are recognized for lymphoma, with the exception of viruses, immunosuppression or smoking, which are associated with specific lymphoma subtypes. Associations between lymphoma and TCE exposure are based on groupings of several lymphoma subtypes. Three of the six lymphoma case-control studies adjusted for age, sex and smoking in statistical analyses (Miligi et al., 2006; Seidler et al., 2007; Wang et al., 2009), the other three case-control studies presented only unadjusted estimates of the odds ratio.

Animal studies describing rates of lymphomas and/or leukemias in relation to TCE exposure (NTP, 1990, 1988; NCI, 1976; Henschler et al., 1980, 1984; Maltoni et al., 1986, 1988) are available. Henschler et al. (1980) reported statistically significant increases in lymphomas in female Han:NMRI mice treated via inhalation. While Henschler et al. (1980) suggested these lymphomas were of viral origin specific to this strain, subsequent studies reported increased lymphomas in female B6C3F1 mice treated via corn oil gavage (NTP, 1990) and leukemias in male Sprague-Dawley and female August rats (Maltoni et al., 1986; NTP, 1988). However, these tumors had relatively modest increases in incidence with treatment, and were not reported to be increased in other studies.

4.7. RESPIRATORY TRACT TOXICITY AND CANCER

4.7.1. Epidemiologic Evidence

4.7.1.1. *Chronic Effects: Inhalation*

Two reports of a study of 1,091 gun-manufacturing workers are found on noncancer pulmonary toxicity (Cakmak et al., 2004; Saygun et al., 2007). A subset of these workers (n = 411) had potential exposure to multiple organic solvents including toluene, acetone, butanol, xylene, benzene and TCE used to clean gun parts; however, both papers lacked information on exposure concentration. Mean exposure duration in Cakmak et al. (2004) was 17 years (SD = 7.9) for nonsmokers and 16 years (SD = 7.1) for smokers. Cakmak et al. (2004) indicated effects of smoking and exposure to solvents, with smoking having the most important effect on asthma-related symptoms (smoking, OR = 2.8, 95% CI: 2.0, 3.8; solvent exposure, OR = 1.4, 95% CI: 1.1, 1.9). Similarly, smoking, but not solvent exposure, was shown as a statistically significantly predictor of lung function decrements. Saygun et al. (2007) reported on a five year follow-up of 393 of the original 1,091 subjects, 214 of who were exposed to solvents. Of the 393 original subjects, the prevalence of definitive asthma symptoms, a more rigorous definition than used by Cakmak et al. (2004), was 3.3% among exposed and 1.1% among nonexposed subjects, $p > 0.05$. Saygun et al. (2007) presents observations on lung function tests for 697 current workers, a group which includes the 393 original study subjects. Smoking, but not solvent exposure, was a predictor of mean annual forced expiratory volume (FEV_1) decrease.

4.7.1.2. *Cancer*

Cancers of the respiratory tract including the lung, bronchus, and trachea are examined in 25 cohort, community studies and case-control studies of TCE. Twelve of the 25 studies approached standards of epidemiologic design and analysis identified in the review of the epidemiologic body of literature on TCE and cancer (see Appendix B; Siemiatycki, 1991;

Axelson et al., 1994; Greenland et al., 1994; Anttila et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999, 2006; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Radican et al., 2008). Cancers at other sites besides lung, bronchus, and trachea in the respiratory system are more limitedly reported in these studies. Some information is available on laryngeal cancer; however, only 9 of the 16 occupational cohort studies providing information on lung cancer also reported findings for this site. Case-control studies of lung or laryngeal cancers and occupational title or organic solvent exposure were found in the literature. Two case-control studies of lung cancer, one population-based and the other nested within a cohort, were of TCE exposure specifically. Lung and laryngeal cancer risk ratios reported in cohort, community and case-control studies are found in Table 4-70.

Table 4-70. Selected results from epidemiologic studies of TCE exposure and lung cancer

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				
	Any exposure to TCE	Not reported		Zhao et al., 2005
	Low cumulative TCE score	1.00[a]	43	
	Medium cumulative TCE score	1.36 (0.86, 2.14)	35	
	High TCE score	1.11 (0.60, 2.06)	14	
	p for trend	0.60		
All employees at electronics factory (Taiwan)		1.07 (0.72, 1.52)	30	Chang et al., 2005
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure, all subjects	1.4 (1.32, 1.55)	632	
	Any exposure, males	1.4 (1.28, 1.51)	559	
	Any exposure, females	1.9 (1.48, 2.35)	73	
	Employment duration			
	<1 yr	1.7 (1.46, 1.93)	209	
	1−4.9 yrs	1.3 (1.16, 1.52)	218	
	≥5 yrs	1.4 (1.23, 1.63)	205	

Table 4-70. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Biologically-monitored Danish workers				Hansen et al., 2001
	Any TCE exposure, males	0.8 (0.5, 1.3)	16	
	Any TCE exposure, females	0.7 (0.01, 3.8)	1	
	Cumulative exposure (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			
	Mean concentration (Ikeda)	Not reported		
	<4 ppm			
	4+ ppm			
	Employment duration	Not reported		
	<6.25 yr			
	≥6.25 yr			
Aircraft maintenance workers (Hill Air Force Base, UT)				Blair et al., 1998
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.0 (0.6, 2.0)	24	
	5−25 ppm-yr	0.8 (0.4, 1.6)	11	
	>25 ppm-yr	0.8 (0.4, 1.7)	15	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr		1	
	5−25 ppm-yr		1	
	>25 ppm-yr		1	
Biologically-monitored Finnish workers				Anttila et al., 1995
	All subjects	0.92 (0.59, 1.35)	25	
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	1.02 (0.58, 1.66)	16	
	6+ ppm	0.83 (0.33, 1.71)	7	
Biologically-monitored Swedish workers				Axelson et al., 1994
	Any TCE exposure, males	0.69 (0.31, 1.30)	9	
	Any TCE exposure, females	Not reported		
Cohort and PMR -mortality				
Computer manufacturing workers (IBM), NY				Clapp and Hoffman 2008
	Males	1.03 (0.71, 1.42)	35	
	Females	0.95 (0.20, 2.77)	3	

Table 4-70. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aerospace workers (Rocketdyne)				
	Any TCE (utility or engine flush workers)	1.24 (0.92, 1.63)	51	Boice et al., 2006
	Engine flush—duration of exposure			
	Referent	1.0[a]	472	
	0 yr (utility workers with TCE exposure)	0.5 (0.22, 1.00)	7	
	<4 yrs	0.8 (0.50, 1.26)	27	
	≥4 yrs	0.8 (0.46, 1.41)	24	
	Any exposure to TCE	Not reported		Zhao et al., 2005
	Low cumulative TCE score	1.00[a]	99	
	Medium cumulative TCE score	1.05 (0.76, 1.44)	62	
	High TCE score	1.02 (0.68, 1.53)	33	
	p for trend	0.91		
View-Master employees				ATSDR, 2004
	Males	0.81 (0.42, 1.42)[b]	12	
	Females	0.99 (0.71, 1.35)[b]	41	
United States uranium-processing workers (Fernald)				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration[c]	Not reported		
	Moderate TCE exposure, >2 yrs duration[c]	Not reported		
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure	0.76 (0.60, 0.95)	78	
	Routine-intermittent exposure[a]	Not reported	173	
	Duration of exposure			
	0 yrs	1.0	288	
	<1 yr	0.85 (0.65, 1.13)	66	
	1−4 yrs	0.98 (0.74, 1.30)	63	
	≥5 yrs	0.64 (0.46, 0.89)	44	
	Trend test	$p < 0.05$		
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	1.10 (0.89, 1.34)	97	
	Low intensity (<50 ppm)	1.49 (1.09, 1.99)	45	
	High intensity (>50 ppm)	0.90 (0.67, 1.20)	52	
	TCE subcohort (Cox Analysis) [b]			
	Never exposed	1.00[a]	291	
	Ever exposed	1.14 (0.90, 1.44)	97	
	Peak			
	No/Low	1.00[a]	324	

Table 4-70. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Medium/High	1.07 (0.82, 1.40)	64	
	Cumulative			
	Referent	1.00[a]	291	
	Low	1.47 (1.07, 2.03)	45	
	High	0.96 (0.72, 1.29)	52	
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al., 1998
	TCE subcohort			
	Any TCE exposure	0.9 (0.6, 1.3)[a]	109	
	Males, cumulative exposure			
	0	1.0[a]	51	
	<5 ppm-yr	1.0 (0.7, 1.6)	43	
	5−25 ppm-yr	0.9 (0.5, 1.6)	23	
	>25 ppm-yr	1.1 (0.7, 1.8)	38	
	Females, Cumulative exp			
	0	1.0[a]	2	
	<5 ppm-yr	0.6 (0.1, 2.4)	2	
	5−25 ppm-yr	0.6 (0.1, 4.7)	11	
	>25 ppm-yr	0.4 (0.1, 1.8)	2	
	TCE subcohort			Radican et al., 2008
	Any TCE exposure	0.83 (0.63, 1.08)	166	
	Males, cumulative exposure	0.91 (0.67, 1.24)	155	
	0	1.0[a]	66	
	<5 ppm-yr	0.96 (0.67, 1.37)		
	5−25 ppm-yr	0.71 (0.46, 1.11)	31	
	>25 ppm-yr	1.00 (0.69, 1.45)	58	
	Females, cumulative exposure	0.53 (0.27, 1.07)	11	
	0	1.0[a]		
	<5 ppm-yr	0.69 (0.27, 1.77)	5	
	5−25 ppm-yr	0.65 (0.16, 2.73)	2	
	>25 ppm-yr	0.39 (0.14, 1.11)	4	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	TCE-exposed workers	1.38 (0.55, 2.86)	7	
	Unexposed workers	1.06 (0.34, 2.47)	5	
Deaths reported to GE pension fund (Pittsfield, MA)		1.01 (0.69, 1.47)[d]	139	Greenland et al., 1994
U.S. Coast Guard employees				Blair et al., 1989
	Marine inspectors	0.52 (0.31, 0.82)	18	
	Noninspectors	0.81 (0.55, 1.16)	30	

Table 4-70. Selected results from epidemiologic studies of TCE exposure and lung cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aircraft manufacturing employees (Italy)				Costa et al., 1989
	All employees	0.99 (0.73, 1.32)	99	
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects	0.80 (0.68, 0.95)	138	
Lamp manufacturing workers (GE)		0.58 (0.27, 1.27)	6	Shannon et al., 1988
Rubber industry workers (Ohio)		0.64 ($p > 0.05$) [c]	11	Wilcosky et al., 1984
Case-control studies				
Population of Montreal, Canada				Siemiatycki et al., 1991
	Any TCE exposure	0.9 (0.6, 1.5)[e]	21	
	Substantial TCE exposure	0.6 (0.3, 1.2)[e]	9	
Geographic based studies				
Two study areas in Endicott, NY		1.28 (0.99, 1.62)	68	ATSDR, 2006
Residents of 13 census tracts				Morgan and Cassidy, 2002
	In Redland, CA	0.71 (0.61, 0.81)[f]	356	
Iowa residents with TCE in water supply				Isacson et al., 1985
	Males			
	<0.15 µg/L	343.1[g]	1,181	
	≥0.15 µg/L	345.7[g]	299	
	Females			
	<0.15 µg/L	58.7[g]	289	
	≥0.15 µg/L	47.8[g]	59	

[a]Internal referents, workers not exposed to TCE.
[b]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex, and decade (Environmental Health Strategies, 1997).
[c] Odds ratio from nested case-control study.
[d]Odds ratio from nested case-control analysis.
[e]90% confidence interval.
[f]99% confidence interval.
[g]Average annual age-adjusted incidence (per 100,000).

Lung cancer relative risks were reported in 11 of 12 cohort studies of aircraft manufacturing, aircraft maintenance, aerospace, and metal workers, with potential exposure to TCE as a degreasing agent, and in occupational cohort studies employing biological markers of TCE exposures. All 11 studies had a high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Axelson et al., 1994; Greenland et al., 1994; Anttila et al., 1995; Blair et al.,

1998; Morgan et al., 1998; Boice et al., 1999, 2006; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Radican et al., 2008). Lung cancer risks were not reported for Fernald uranium processing workers with potential TCE exposure (Ritz, 1999), a study of less weight than the other 11 studies.. The incidence study of Raaschou-Nielsen et al. (2003) was the largest cohort, with 40,049 subjects identified as potentially exposed to TCE in several industries (primarily, in the iron/metal and electronic industries), including 14,360 of whom had presumably higher level exposures to TCE. The study included 632 lung cancer cases and reported a 40% elevated incidence in TCE exposed males and females combined (95% CI: 1.32, 1.55), with no exposure duration gradient. The 95% confidence intervals in other studies of lung cancer incidence included a risk ratio of 1.0 (Axelson et al., 1994; Anttila et al., 1995; Blair et al., 1998; Hansen et al., 2001; Zhao et al., 2005). Lung cancer mortality risks in studies of TCE exposure to aircraft manufacturing, aircraft maintenance, and aerospace workers included a relative risk of 1.0 in their 95% confidence intervals (Boice et al., 2006; Zhao et al., 2005; Morgan et al., 1998; Blair et al., 1998). Boice et al. (1999) observed a 24% decrement (95% CI: 0.60, 0.95) for subjects with routine TCE exposure. Exposure-response analyses using internal controls (unexposed subjects at the same company) showed a statistically significant decreasing trend between lung cancer risk and routine or intermittent TCE exposure duration. The routine or intermittent category is broader and includes more subjects with potential TCE exposure.

The population studied by Garabrant et al. (1998), ATSDR (2004) and Chang et al. (2005) are all employees (white- and blue-collar) at a manufacturing facility or plant with potential TCE exposures. Garabrant et al. (1988) observed a 20% deficit in lung cancer mortality (95% CI: 0.68, 0.95) in their study of all employees working for 4 or more years at an aircraft manufacturing company. Blair et al. (1989), a study of Coast Guard marine inspectors with potential for TCE exposure but lacking assessment to individual subjects, observed a 48% deficit in lung cancer mortality (95% CI: 0.31, 0.82). Confidence intervals (95% CI) in Costa et al. (1989), Chang et al. (2005) and ATSDR (2004) included a risk of 1.0. TCE exposure was not known for individual subjects in these studies. A wide potential for TCE exposure is likely ranging from subjects with little to no TCE exposure potential to those with some TCE exposure potential. Exposure misclassification bias, typically considered as a negative bias, is likely greater in these studies compared to studies adopting more sophisticated exposure assessment approaches, which are able to assign quantitative exposure metrics to individual study subjects. All three studies were of lower likelihood for TCE exposure, in addition to limited statistical power and other design limitations, and these aspects, in addition to potential exposure misclassification bias were alternative explanations of observed findings.

One population case-control study examined the relationship between lung cancer and TCE exposure (Siemiatycki et al., 1991) with risk ratios of 0.9 (95% CI: 0.6, 1.5) for any TCE exposure and 0.6 (95% CI: 0.3, 1.2) for substantial TCE exposure after adjustment for cigarette smoking. TCE exposure prevalence in cases in this study was 2.5% for any exposure. Only 1% had "substantial" (author's term) exposure, limiting the sensitivity of this study. Relative risks above 2.0 could only be detected with sufficient (80%) statistical power. The finding of no association of lung cancer with TCE exposure, therefore, is not surprising. One nested case-control study of rubber workers observed a smoking unadjusted risk of 0.64 (95% CI: not presented in paper) in those who had >1 year cumulative exposure to TCE (Wilcosky et al., 1984).

Three geographic based studies reported lung cancer incidence or mortality risks for drinking water contamination with TCE (Isacson et al., 1985; Morgan and Cassidy, 2002; ATSDR, 2006). Morgan and Cassidy (2002) observed a relative risk of 0.71 (99% CI: 0.61, 0.81) for lung cancer among residents of Redlands County, CA, whose drinking water was contaminated with TCE and perchlorate. However, ATSDR (2006) reported a 28% increase (95% CI: 0.99, 1.62) in lung cancer incidence among residents living in a area in Endicott, NY, whose drinking water was contaminated with TCE and other solvents. No information on smoking patterns is available for individual lung cancer cases as identified by the New York State Department of Health (NYS DOH) for other cancer cases in this study (ATSDR, 2008). Isacson et al. (1985) presented lung cancer age-adjusted incidence rates for Iowa residents by TCE level in drinking water supplies and did not observe an exposure-response gradient. Exposure information is inadequate in all three of these studies, with monitoring data, if available, based on few samples and for current periods only, and no information on water distribution, consumption patterns, or temporal changes. Thus, TCE exposure potential to individual subjects was not known with any precision, introducing misclassification bias, and greatly limiting their ability to inform evaluation of TCE and lung cancer.

Laryngeal cancer risks are presented in a limited number of cohort studies involving TCE exposure. No case-control or geographic based studies of TCE exposure were found in the published literature. All but one of the cohort studies providing information on laryngeal cancer observed less that 5 incident cases or deaths. Accordingly, these studies are limited for examining the relationship between TCE exposure and laryngeal cancer. Risk ratios for laryngeal cancer are found in Table 4-71.

Table 4-71. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer

1

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort studies—incidence				
Aerospace workers with TCE exposure		Not reported		Zhao et al., 2005
Danish blue-collar worker w/TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure, males	1.2 (0.87, 1.52)	53	
	Any exposure, females	1.7 (0.33, 4.82)	3	
	Employment duration	Not reported		
	<1 yr			
	1−4.9 yrs			
	≥5 yrs			
Biologically-monitored Danish workers				Hansen et al., 2001
	Any TCE exposure, males	1.1 (0.1, 3.9)	2	
	Any TCE exposure, females		0 (0.1 exp)	
	Cumulative exposure (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			
	Mean concentration (Ikeda)	Not reported		
	<4 ppm			
	4+ ppm			
	Employment duration	Not reported		
	<6.25 yr			
	≥6.25 yr			
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al., 1998
	TCE subcohort			
	Any exposure	Not reported		
	Males, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5−25 ppm-yr			
	>25 ppm-yr			
	Females, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5−25 ppm-yr			
	>25 ppm-yr			

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Table 4-71. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Biologically-monitored Finnish workers		Not reported		Anttila et al., 1995
	Mean air-TCE (Ikeda extrapolation from U-TCA)	Not reported		
	<6 ppm			
	6+ ppm			
Biologically-monitored Swedish workers				Axelson et al., 1994
	Any TCE exposure, males	1.39 (0.17, 5.00)	2	
	Any TCE exposure, females	Not reported		
Cohort and PMR -Mortality				
Computer manufacturing workers (IBM), NY		Not reported		Clapp and Hoffman (2008)
Aerospace workers (Rocketdyne)				
	Any TCE (utility or engine flush workers)	1.45 (0.18, 5.25)	2	Boice et al., 2006
	Engine flush—duration of exposure	Not reported		
	Referent			
	0 yr (utility workers with TCE exposure)			
	<4 yrs			
	≥4 yrs			
	Any exposure to TCE	Not reported		Zhao et al., 2005
View-Master employees		Not reported		ATSDR, 2004
	Males			
	Females			
All employees at electronic factory (Taiwan)				Chang et al., 2003
	Males		0 (0.90 exp)	
	Females	0	0 (0.23 exp)	
United States uranium-processing workers (Fernald)				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration[4]	Not reported		
	Moderate TCE exposure, >2 yrs duration[4]	Not reported		
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure	1.10 (0.30, 2.82)	4	
	Routine-intermittent exposure	Not reported		

Table 4-71. Selected results from epidemiologic studies of TCE exposure and laryngeal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	Not reported		
	Low intensity (<50 ppm)			
	High intensity (>50 ppm)			
	Peak	Not reported		
	No/low			
	Medium/high			
	Cumulative	Not reported		
	Referent			
	Low			
	High			
Aircraft maintenance workers (Hill Air Force Base, Utah)				Blair et al., 1998
	TCE subcohort	Not reported		
	Males, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5−25 ppm-yr			
	>25 ppm-yr			
	Females, cumulative exposure	Not reported		
	0			
	<5 ppm-yr			
	5−25 ppm-yr			
	>25 ppm-yr			
Cardboard manufacturing workers in Arnsburg, Germany		Not reported		Henschler et al., 1995
Deaths reported to GE pension fund (Pittsfield, MA)		Not examined		Greenland et al., 1994
U. S. Coast Guard employees				Blair et al., 1989
	Marine inspectors	0.57 (0.01, 3.17)	1	
	Noninspectors	0.58 (0.01, 3.20)	1	
Aircraft manufacturing employees (Italy)				Costa et al., 1989
	All employees	0.27 (0.03, 0.98)	2	
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects		0 (7.41 exp)	

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In summary, studies in humans examining lung and laryngeal cancer and TCE exposure are inconclusive and do not support either a positive or a negative association between TCE exposure and lung cancer or laryngeal cancer. Raaschou-Nielsen et al. (2003), with the largest numbers of lung cancer cases of all studies, was the only one to observe a statistically significantly elevated lung cancer risk with TCE exposure. Raaschou-Nielsen et al. (2003) also noted several factors that may have confounded or biased their results in either a positive or negative direction. This study and other cohort studies, as with almost any occupational study, were not able to control confounding by exposure to chemicals other than TCE (although no such chemical was apparent in the reports). Information available for factors related to socioeconomic status (e.g., diet, smoking, alcohol consumption) was also not available. Such information may positively confound smoking-related cancers such as lung cancer, particularly in those studies, which adopted national rates to derive expected numbers of site-specific cancer, if greater smoking rates were over-represented in blue-collar workers or residents of lower socio-economic status. The finding of a larger risk among subjects with shortest exposure also argues against a causal interpretation for the observed association for all subjects (NRC, 2006).

Four studies reported a statistically significant deficit in lung cancer incidence (Blair et al., 1989; Garabrant et al., 1988; Boice et al., 1999; Morgan and Cassidy, 2002). Absence of smoking information in these studies would introduce a negative bias if the studied population smoked less than the referent population and may partially explain the lung cancer decrements observed in these studies. Morgan and Cassidy (2002) noted the relatively high education high income levels, and high access to health care of subjects in this study compared to the averages for the county as a whole, likely leading to a lower smoking rate compared to their referent population. Garabrant et al. (1988) similarly attributed their observations to negative selection bias introduced when comparison is made to national mortality rates, also known as a "healthy worker effect." The statistically significant decreasing trend in Boice et al. (1999) with exposure duration to intermittent or routine exposure may reflect a protective effect between TCE and lung cancer. The use of internal controls in this analysis reduces bias associated with use of an external population who may have different smoking patterns than an employed population. However, the exposure assessment approach in this study is limited due to inclusion of subjects identified with intermittent TCE exposure (i.e., workers who would be exposed only during particular shop runs or when assisting other workers during busy periods) (Boice et al., 1999). The Boice et al. (1999) analysis is based on twice as many lung cancer deaths (i.e., 173 lung cancer deaths) among subjects with routine or intermittent TCE exposure compared to only routinely exposed subjects (78 deaths). Subjects identified as intermittently exposed are considered as having a lower exposure potential than routinely exposed subject and their

inclusion in exposure-response analyses may introduce exposure misclassification bias. Such bias is a possible explanation for the decreasing trend observation, particularly if workers with lower potential for TCE exposure have longer exposure (employment) durations.

Thus, a qualitative assessment suggests the epidemiological literature on respiratory cancer and TCE is quite limited and has sufficient power to detect only large relative risks. These studies can only rule out risks of a magnitude of 2.0 or greater for lung cancer and relative risks greater than 3.0 or 4.0 for laryngeal cancer for exposures to studied populations. Therefore, the database is limited in its ability to detect lung cancer associated with TCE exposure, especially if the magnitude of response is similar to those observed for other endpoints.

4.7.2. Laboratory Animal Studies

4.7.2.1. *Respiratory Tract Animal Toxicity*

Limited studies are available to determine the effects of TCE exposure on the respiratory tract (summarized in Table 4-72). Many of these studies in mice have examined acute effects following intraperitoneal administration at relatively high TCE doses. However, effects on the bronchial epithelium have been noted in mice and rats with TCE administered via gavage, with doses 1,000 mg/kg/d and higher reported to cause rales and dyspnea (Narotsky et al., 1995) and pulmonary vasculitis (NTP, 1990) in rats. Mice appear to be more sensitive than rats to histopathological changes in the lung via inhalation; pulmonary effects are also seen in rats with gavage exposure. It is difficult to compare intraperitoneal to oral and inhalation routes of exposure given the risk of peritonitis and paralytic ileus. Any inflammatory response from this route of administration can also affect the pulmonary targets of TCE exposure such as the Clara cells.

This section reviews the existing literature on TCE, and the role of the various TCE metabolites in TCE-induced lung effects. The most prominent toxic effect reported is damage to Clara cells in mouse lung. The nonciliated, columnar Clara cells comprise the majority of the bronchiolar and terminal bronchiolar epithelium in mice, and alveolarType I and Type II cells constitute the alveolar epithelium. These cells have been proposed as a progenitor of lung adenocarcinomas in both humans and mice (Kim et al., 2005). Long-term studies have not focused on the detection of pulmonary adenoma carcinomas but have shown a consistently positive response in mice but not rats. However, chronic toxicity data on noncancer effects is very limited.

Table 4-72. Animal toxicity studies of trichloroethylene

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Green et al., 1997	CD-1 mice (F)	Inhalation	450-ppm, 6 h/d, 5 d with 2 d break then 5 more days; sacrificed 18 h after 1, 5, 6, or 10 exposures	5/group	Increased vacuolation and proliferation of Clara cells caused by accumulation of chloral.
Forkert and Forkert, 1994	CD-1 mice (M)	Intraperitoneal injection	2,000 mg/kg in corn oil (0.01 mL/g BW); sacrificed 15, 30, 60 and 90 d after single exposure	10/group	Increased fibrotic lesions, with early signs visible at 15 d postexposure.
Villaschi et al., 1991	BC3F1 mice (M)	Single inhalation	30 min 500, 1,000, 2,000, 3,500, and 7,000 ppm; sacrificed 2 h, 24 h, 2, 5, or 7 d post exposure	3/group	Increased vacuolation and proliferation of nonciliated bronchial cells. Injury was maximal at 24 h with some repair occurring between 24 h and 48 h.
Odum et al., 1992	CD-1 mice (F)	Inhalation	6 h/d; separate repeated study in mice: 450 ppm for 6 h/d, 5 d/wk for 2 wks; sacrificed 24 h after exposure; repeat study sacrificed at 2, 5, 6, 8, 9, 12, or 13 d; mice: 20, 100, 200, 450, 1,000, or 2,000 ppm	4/group	Dose-dependent increase in Clara cell vacuolation in mice after a single exposure, resolved after 5 d repeated exposures but recurred following a 2-d break from exposure. Changes accompanied by decrease in CYP activity in mice. Exposure to chloral alone demonstrated similar response as TCE exposure in mice. No changes were seen in rats.
	Alpk APfSD rats (F)	Inhalation	6 h/d; repeat study sacrificed at 2, 5, 6, 8, 9, 12, or 13 d; rats: 500, or 1,000 ppm	4/group	
Kurasawa, 1988 (translation)	Ethanol-treated (130) and nontreated (110) Wistar rats (M)	Inhalation	500, 1,000, 2,000, 4,000, and 8,000 ppm for 2 h; sacrificed 22 h after exposure	10/group	TCE exposure resulted in highly selective damage to Clara cells that occurred between 8 and 22 h after the highest exposure with repair by 4 wks post exposure.

Table 4-72. Animal toxicity studies of trichloroethylene (continued)

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
Forkert et al., 2006	CD-1 mice (M); wild-type (mixed 129/Sv and C57BL) and CYP2E1-null mice (M)	Intraperitoneal injection	500, 750, and 1,000 mg/kg in corn oil; for inhibition studies mice pretreated with 100 mg/kg diallyl sulfone; for immunoblotting, 250, 500, 750, and 1,000 mg/kg; for PNP hydroxylation, 50, 100, 250, 500, 750, and 1,000 mg/kg; sacrificed 4 h after exposure	4/group	TCE bioactivation by CYP2E1 and/or 2F2 correlated with bronchiolar cytotoxicity in mice.
Forkert et al., 1985	CD-1 mice (M)	Intraperitoneal injection	2,000, 2,500 or 3,000 mg/kg in mineral oil; sacrificed 24 h postexposure for dose response; time course sacrificed 1, 2, 12, and 24 h postexposure	10/group	Clara cell injury was increased following exposure at all doses tested; time course demonstrated a rapid and marked reduction in pulmonary microsomal cytochrome P450 content and aryl hydrocarbon hydroxylase activity. Alveolar Type II cells were also affected.
Forkert and Birch, 1989	CD-1 mice (M)	Intraperitoneal injection	2,000 mg/kg in corn oil; sacrificed 1, 2, 4, 8, 12, and 24 h postexposure	10/group	Necrotic changes seen in Clara cells as soon as 1 h postexposure; increased vacuolation was seen by 4 h postexposure; covalent binding of TCE to lung macromolecules peaked at 4 h and reached a plateau at 12 and 24 h post exposure.
Stewart et al., 1979; Le Mesurier et al., 1980	Wistar Rats (F)	Inhalation (whole body chamber)	30 min, 48.5 g/m^3 (9,030 ppm); sacrificed at 5 and 15 d postexposure	5/group	Decreased recovery of pulmonary surfactant (dose-dependent).
Lewis, 1984	Mice	Inhalation (Pyrex bell jars)	10,000 ppm, 1−4 h daily for 5 consecutive days; sacrificed 24 h after last exposure	~28/group	Increased vacuolation and reduced activity of pulmonary mixed function oxidases.
Scott et al., 1988	CD-1 mice (M)	Intraperitoneal injection	single injection of 2,500−3,000 mg/kg, sacrificed 24 h postexposure	4/group	Clara cells were damaged and exfoliated from the epithelium of the lung.

Table 4-72. Animal toxicity studies of trichloroethylene (continued)

Reference	Animals (sex)	Exposure route	Dose/exposure concentration	Exposed	Results
NTP, 1990	F344 rats (M,F) B6C3F1 mice (M,F)	Gavage	Male rats: 0, 125, 250, 500, 1,000, and 2,000 mg/kg BW (corn oil); female rats: 0, 62.5, 125, 250, 500 or 1,000 mg/kg BW (corn oil); Mice: 0, 375, 750, 1,500, 3,000, and 6,000 mg/kg BW (corn oil); dosed 5d/w for 13 wks	10/group	Increased pulmonary vasculitis in the high-dose groups of male and female rats (6/10 group as compared to 1/10 in controls). No pulmonary effects described in mice at this time point.
Prendergast et al., 1967	Sprague-Dawley or Long-Evans rats; Hartley Guinea pigs; New Zealand albino rabbits; beagle dogs; squirrel monkeys (sex not given for any species)	Inhalation	730 ppm for 8 h/d, 5 d/w, 6 wks or 35 ppm for 90 d constant	Rats (15); guinea pigs (15); rabbit (3); dog (2); monkey (3)	No histopathological changes observed, although rats were described to show a nasal discharge in the 6 wk study. No quantification was given.
Narotsky et al., 1995	F344 rats (F)	Gavage	0, 1,125, 1,500 mg/kg/d	21, 16, or 17 per group	Rales and dyspnea were observed in the TCE high-dose group; two females with dyspnea subsequently died.

4.7.2.1.1. ***Acute and short-term effects: inhalation.*** Relatively high-dose single and multiple inhalation exposures to TCE result in dilation of endoplasmic reticulum and vacuolation of nonciliated (Clara) cells throughout the bronchial tree in mice. A single study in rats reported similar findings. In mice, single exposure experiments show vacuolation at all dose levels tested with the extent of damage increasing with dose. Villaschi et al. (1991) reported similar degrees of vacuolation in B6C3F1 mice (3/group) at 24 hours after the start of exposure across all tested doses (500, 1,000, 2,000, 3,500, and 7,000 ppm, 30 minutes), with the percentage of the nonciliated cells remaining vacuolated at 48 hours increasing with dose. Clara cell vacuolation was reported to be resolved 7 days after single 30 minute exposure to TCE. Odum et al. (1992) reported that, when observed 24 hours after the start of 6 hours exposure, the majority of Clara cells in mice were unaffected at the lowest dose of 20 ppm exposures, while marked vacuolation was observed at 200 ppm (no quantitative measures of damage given and only 3 animals per group were examined).

In rats, Odum et al. (1992) reported no morphological changes in the female Alpk APfSD rat epithelium after 6 hours exposure (500 or 1,000 ppm) when observed 24 hours after the start of exposure (n = 3/group). However, Kurasawa reported pronounced dose-related morphological changes in Clara cells at the highest dose (8,000 ppm) for 2 hours in Wistar rats (n = 10 per group). At 500 and 1,000 ppm, slight dilation of the apical surface was reported, but morphological measurements (the ratio of the lengths of the apical surface to that of the base line of apical cytoplasm) were not statistically-significantly different from controls. From 2,000 to 8,000 ppm, a progressively increasing flattening of the apical surface was observed. In addition, at 2,000 ppm, slight dilation of the smooth endoplasmic reticulum was also observed, with marked dilation and possible necrosis at 8,000 ppm. Kurasawa (1988) also examined the time-course of Clara cell changes following a single 8,000-ppm exposure, reporting the greatest effects at 1 day to 1 week, repair at 2 weeks, and nearly normal morphology at 4 weeks. The only other respiratory effect that has been reported from one study in rats exposed via inhalation is a reduction in pulmonary surfactant yield following 30 minute exposures at 9,030 ppm for 5 or 15 days (Stewart et al., 1979). Therefore, single inhalation experiments (Villaschi et al., 1991; Odum et al., 1992; Kurasawa, 1988) suggest that the Clara cell is the target for TCE exposure in both rats and mice and that mice are more susceptible to these effects. However, the database is limited in its ability to discern quantitative differences in susceptibility or the nature of the dose-response after a single dose of TCE.

Other experiments examined the effects of several days of TCE inhalation exposure in mice and potential recovery. While single exposures require 1 to 4 weeks for complete recovery, after short-term repeated exposure, the bronchial epithelium in mice appears to either adapt to or

become resistant to damage Odum et al. (1992) and Green et al. (1997) observed Clara cells in mice to be morphologically normal at the end of exposures 6 hours/day for 4 or 5 days. As with single dose experiments, the extent of recovery in multidose exposures may be dose-dependent. Using a very high dose, Lewis et al. (1984) report vacuolation of bronchial epithelial cells after 4 hours/day, but not 1 hours/day, (10,000 ppm) for 5 days in mice. In addition, Odum et al. (1992) reported that the damage to Clara cells that resolved after repeated exposures of 5 days, a sign of adaptation to TCE exposure, returned when exposure was resumed after 2 days.

In rats, only one inhalation study reported in two published articles (Stewart et al., 1979; Le Mesurier et al., 1979) using repeated exposures examined pulmonary histopathology. Interestingly, this study reported vacuolation in Type 1 alveolar cells, but not in Clara cells, after 5 days of exposure to approximately 9,030 ppm for 30 minutes/day (only dose tested). In addition, abnormalities were observed in the endothelium (bulging of thin endothelial segments into the microcirculatory lumen) and minor morphological changes in Type 2 alveolar cells. Although exposures were carried out for 5 consecutive days, histopathology was recorded up to 15 days post exposure, giving cell populations time to recover. Because earlier time points were not examined, it is not possible to discern whether the lack of reported Clara cell damage in rats following repeated exposure is due to recovery or lack of toxicity in this particular experiment.

Although recovery of individual damaged cells may occur, cell proliferation, presumed from labeling index data suggestive of increased DNA synthesis, contributes, at least in part, to the recovery of the bronchial epithelium in mice. Villaschi et al. (1991) observed a dose-dependent increase in labeling index as compared to controls in the mouse lung at 48 hours after a single TCE exposure (30 minutes; 500, 1,000, 2,000, 3,500, 7,000 ppm), which decreased to baseline values at 7 days postexposure. Morphological analysis of cells was not performed, although the authors stated the dividing cells had the appearance of Clara cells. Interestingly, Green et al. (1997) reported no increase in BrdU labeling 24 hours after a single exposure (6 hours 450 ppm), but did see increased BrdU labeling at the end of multiple exposures (1/day, 5 days) while Villaschi et al. (1991) reported increased [^{3}H]Thymidine labeling 2, 5, and 7 days after single 30 minute exposures to 500−7,000 ppm. Therefore, the data for single exposures at 450−500 ppm may be consistent if increased cell proliferation occurred only for a short period of time around 48 hours postexposure, and was thereby effectively washed-out by the longer "averaging time" in the experiments by Green et al. (1997). Also, these contradictory results may be due to differences in methodology. Green et al. (1997) and Villaschi et al. (1991) reported very different control labeling indices (6 and 0%, respectively) while reporting similar absolute labeling indices at 450−500 ppm (6.5 and 5.2%, respectively). The different control values may be a result of substantially-different times over which the label was incorporated: the

mice in Green et al. (1997) were given BrdU via a surgically-implanted osmotic pump over 4 days prior to sacrifice, while the mice in Villaschi et al. (1991) were given a single intraperitoneal dose of [^{3}H]Thymidine 1 hour prior to sacrifice. Stewart et al. (1979) observed no stimulation of thymidine incorporation after daily exposure to TCE (9,000 ppm) for up to 15 days. This study did, however, report a nonstatistically significant reduction in orotate incorporation, an indicator of RNA synthesis, after 15 days, although the data was not shown.

At the biochemical level, changes in pulmonary metabolism, particularly with respect to CYP activity, have been reported following TCE exposure via inhalation or intraperitoneal administration in mice. Odum et al. (1992) reported reduced enzyme activity in Clara cell sonicates of ethoxycoumarin *O*-deethylase, aldrin epoxidation, and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) cytochrome c reductase after 6 hour exposures to 20−2,000 ppm TCE, although the reduction at 20 ppm was not statistically significant. No reduction of GST activity as determined by chlorodinitrobenzene as a substrate was detected. With repeated exposure at 450 ppm, the results were substrate-dependent, with ethoxycoumarin *O*-deethylase activity remaining reduced, while aldrin epoxidation and NADPH cytochrome c reductase activity showing some eventual recovery by 2 weeks. The results reported by Odum et al. (1992) for NADPH cytochrome c reductase were consistent with those of Lewis et al. (1984), who reported similarly reduced NADPH cytochrome c reductase activity following a much larger dose of 10,000 ppm for 1 and 4 hours/day for 5 days in mice (strain not specified). TCE exposure has also been associated with a decrease in pulmonary surfactant. Repeated exposure of female Wistar rats to TCE (9,000 ppm, 30 minutes/day) for 5 or 15 days resulted in a significant decrease in pulmonary surfactant as compared to unexposed controls (Le Mesurier et al., 1980).

4.7.2.1.1.1. ***Acute and short-term effects: intraperitoneal injection and gavage exposure*.** As stated above the intraperitoneal route of administration is not a relevant paradigm for human exposure. A number of studies have used this route of exposure to study the effects of acute TCE exposure in mice. In general, similar lung targets are seen following inhalation or intraperitoneal treatment in mice (Forkert et al., 2006, 1985; Forkert and Birch, 1989; Scott et al., 1988). Inhalation studies generally reported the Clara cell as the target in mice. No lung histopathology from intraperitoneal injection studies in rats is available. Forkert et al. (1985) and Forkert and Birch (1989) reported vacuolation of Clara cells as soon as 1 hour following intraperitoneal administration of a single dose of 2,000 mg/kg in mice. At 2,500 mg/kg, both Forkert et al. (1985) and Scott et al. (1988) reported exfoliation of Clara cells and parenchymal changes, with morphological distortion in alveolar Type II cells and inconsistently observed

minor swelling in Type I cells at 24 hours postexposure. Furthermore, at 3,000 mg/kg, Scott et al. (1988) also reported a significant (85%) decrease in intracellularly stored surfactant phospholipids at 24 hours postexposure. These data indicate that both Clara cells and alveolar Type I and II cells are targets of TCE toxicity at these doses and using this route of administration. Recently, Forkert et al. (2006) reported Clara cell toxicity that showed increased severity with increased dose (pyknotic nuclei, exfoliation) at 500−1,000 mg/kg intraperitoneal doses as soon as 4 hours postexposure in mice. Even at 500 mg/kg, a few Clara cells were reported with pyknotic nuclei that were in the process of exfoliation. Damage to alveolar Type II cells was not observed in this dose range. The study by Scott et al. (1988) examined surfactant phospholipids and phospholipase A2 activity in male CD-1 mice exposed by intraperitoneal injection of TCE (2,500 or 3,000 mg/kg, 24 hours). The lower concentration led to damage to and exfoliation of Clara cells from the epithelial lining into the airway lumen, while only the higher concentration led to changes in surfactant phospholipids. This study demonstrated an increase in total phospholipid content in the lamellar body fractions in the mouse lung.

The study by Narotsky et al. (1995) exposed F344 timed-pregnant rats to TCE (0, 1,125, and 1,500 mg/kg BW) by gavage and examined both systemic toxicity and developmental effects at 14 days postexposure. Rales and dyspnea in the dams were observed in the high-dose group, with two of the animals with dyspnea subsequently dying. The developmental effects observed in this study are discussed in more detail in Section 4.8.

4.7.2.1.1.2. ***Subchronic and chronic effects*****.** There are a few reports of the subchronic and chronic noncancer effects of TCE on the respiratory system from intraperitoneal exposure in mice and from gavage exposure in rats. Forkert and Forkert (1994) reported pulmonary fibrosis in mice 90 days after intraperitoneal administration of a single 2,000 mg/kg dose of TCE. The effects were in the lung parenchyma, not the bronchioles where Clara cell damage has been observed after acute exposure. It is possible that fibrotic responses in the alveolar region occur irrespective of where acute injury occurs. Effects upon Clara cells can also impact other areas of the lung via cytokine regulation (Elizur et al., 2008). Alternatively, the alveolar and/or capillary components of the lung may have been affected by TCE in a manner that was not morphologically apparent in short-term experiments. In addition effects from a single or a few short-term exposures may take longer to manifest. The latter hypothesis is supported by the alveolar damage reported by Odum et al. (1992) after chloral administration by inhalation, and by the adducts reported in alveolar Type II cells by Forkert et al. (2006) after 500−1,000 mg/kg TCE intraperitoneal administration.

As noted previously, rats have responded to short-term inhalation exposures of TCE with Clara cell and alveolar Type I and II effects. After repeated inhalation exposures over 6 weeks (8 hours/day, 5 days/week, 730 ppm) and continuous exposures over 90 days (35 ppm), Prendergast et al. (1967) noted no histopathologic changes in rats, guinea pigs, rabbits, dogs, or monkeys after TCE exposure, but did describe qualitatively observing some nasal discharge in the rats exposed for 6 weeks. The study details in Prendergast et al. (1967) are somewhat limited. Exposed animals are described as "typically" 15 Long-Evans or Sprague-Dawley rats, 15 Hartley guinea pigs, 3 squirrel monkeys, 3 New Zealand albino rabbits, and 2 beagle dogs. Controls were grouped between studies. In a 13-week NTP study in F344/N rats (n = 10/group) exposed to TCE (0−2,000 mg/kg/d 5 days/week) by gavage, pulmonary vasculitis was observed in 6/10 animals of each sex of the highest dose group (2,000 mg/kg/d), in contrast to1/10 in controls of each sex (NTP, 1990).

4.7.2.2. *Respiratory Tract Cancer*

Limited studies have been performed examining lung cancer following TCE exposure (summarized in Table 4-73). TCE inhalation exposure was reported to cause statistically significant increase in pulmonary tumors (i.e., pulmonary adenocarcinomas) in some studies in mice, but not in studies in rats and hamsters. Oral administration of TCE frequently resulted in elevated lung tumor incidences in mice, but not in any tested species was there a statistically significant increase. This section will describe the data regarding TCE induction of pulmonary tumors in rodent models. The next sections will consider the role of metabolism and potential MOAs for inhalation carcinogenicity, primarily in mice.

4.7.2.2.1. ***Inhalation.*** There are three published inhalation studies examining the carcinogenicity of TCE at exposures from 0−600 ppm, two of which reported statistically significantly increased lung tumor incidence in mice at the higher concentrations (Fukuda et al., 1983; Maltoni et al., 1986, 1988; Henschler et al., 1980). Rats and hamsters did not show an increase in lung tumors following exposure.

Table 4-73. Animal carcinogenicity studies of trichloroethylene

Reference	Animals (sex)	Exposure route	Dose/exp conc (stabilizers, if any)	Pulmonary tumor incidences	
				Benign+malignant	Malignant only
Fukuda et al., 1983	ICR mice (F) S-D rats (F)	Inhalation, 7 h/d, 5 d/wk, 104 wk, hold until 107 wk	0, 50, 150, or 450 ppm (epichlorohydrin)	Mice: 6/49, 5/50, 13/50, 11/46; Rats: 0/50, 0/50, 1/47, 1/51	Mice: 1/49; 3/50; 8/50*; 7/46*; Rats: none
Maltoni et al., 1986, 1988	S-D rats (M, F) Swiss mice (M, F) B6C3F1 mice (M, F)	Inhalation, 7 h/d, 5 d/wk, 104 wk, hold until death	0, 100, 300, or 600 ppm	Rats: 0/280, 0/260, 0/260, 0/260; Swiss Mice: M: 10/90, 11/90, 23/90*, 27/90**; F: 15/90, 15/90, 13/90, 20/90; B6C3F1 Mice: M: 2/90, 2/90, 3/90, 1/90; F: 4/90, 6/90, 7/90, 15/90*;	Rats: 0/280, 0/260, 0/260, 0/260; Swiss Mice: M: 0/90, 0/90, 0/90, 1/90; F: 2/90, 0/90, 0/90, 2/90; B6C3F1 Mice M: 0/90, 0/90, 0/90, 0/90; F: 0/90, 1/90, 0/90, 0/90;
Henschler et al., 1980	Wistar rats (M, F) Syrian hamsters (M, F) NMRI mice	Inhalation, 6 h/d, 5 d/wk, 78 wks, hold until 130 wk (mice and hamsters) or 156 wk (rats)	0, 100, or 500 ppm (triethanolamine)	Rats: M: 1/29, 1/30, 1/30; F: 0/28; 1/30; 0/30; Hamsters: 0/60, 0/59, 0/60; Mice: M: 1/30, 3/29, 1/30; F: 3/29, 0/30,1/28	Rats: M: 1/29, 1/30, 1/30; F: 0/28; 1/30; 0/30; Hamsters: 0/60, 0/59, 0/60; Mice: M: 5/30, 3/29, 1/30; F: 1/29, 3/30,0/28
Henschler et al., 1984	Swiss mice (M, F)	Gavage, 5/wk, 72 wk hold 104 wk	2.4 g/kg BW (M), 1.8 g/kg BW (F) all treatments; (control, triethanolamine, industrial, epichlorohydrin, 1,2-epoxybutane, both)	Male: 18/50, 17/50, 14/50, 21/50, 15/50, 18/50; Female: 12/50, 20/50, 21/50, 17/50, 18/50, 18/50	Male: 8/50, 6/50, 7/50, 5/50, 7/50, 7/50; Female: 5/50, 11/50, 8/50, 3/50, 7/50, 7/50
Van Duuren et al., 1979	Swiss mice (M, F)	Gavage, 1/wk, 89 wk	0 or 0.5 mg (unknown)	0/30 for all groups	0/30 for all groups
NCI, 1976	Osborne-Mendel rats (M, F) B6C3f1 mice (M, F)	Gavage, 5/wk, 78 wk, hold until 110 wk (rats) or 90 wk (mice)	Rats: TWA: 0, 549, or 1,097 mg/kg Mice: TWA: M: 0, 1,169, or 2,339 mg/kg; F: 0, 869, or 1,739 mg/kg (epoxybutane, epichlorohydrin)	Rats: M: 1/20, 0/50, 0/50; F: 0/20, 1/47, 0/50 Mice: M: 0/20, 5/50, 2/48; F: 1/20, 4/50, 7/47	Rats: M: 0/20, 0/50, 0/50; F: 0/20, 1/47, 0/50 Mice: M: 0/20, 0/50, 1/48; F: 0/20, 2/50, 2/47

Table 4-73. Animal carcinogenicity studies of trichloroethylene (continued)

Reference	Animals (sex)	Exposure route	Dose/exp conc (stabilizers, if any)	Pulmonary tumor incidences	
				Benign+malignant	Malignant only
NTP, 1988	ACI, August, Marshall, Osborne-Mendel rats	Gavage, 1/d, 5 d/wk, 103 wk	0, 500, or 1,000 mg/kg (diisopropylamine)	ACI M: 1/50, 4/47, 0/46; F: 0/49, 2/47, 2/42 August M: 1/50, 1/50, 0/49; F: 1/50, 1/50, 0/50 Marshall M: 3/49, 2/50, 2/47; F: 3/49, 3/49, 1/46 Osborne-Mendel M: 2/50, 1/50, 1/50; F: 0/50, 3/50, 2/50	ACI M: 1/50, 2/47, 0/46; F: 0/49, 1/47, 2/42 August M: 0/50, 1/50, 0/49; F: 1/50, 0/50, 0/50 Marshall M: 3/49, 2/50, 2/47; F: 3/49, 3/49, 1/46 Osborne-Mendel M: 1/50, 1/50, 0/50; F: 0/50, 3/50, 1/50
NTP, 1990	F344 rats (M, F) B6C3F1 mice (M, F)	Gavage, 1/day, 5 days/wk, 103 wk	Mice: 0 or 1,000 mg/kg Rats: 0, 500, 1,000 mg/kg	Mice: M: 7/49, 6/50; F: 1/48, 4/49 Rats: M: 4/50, 2/50, 3/49; F: 1/50, 1/49, 4/50	Mice: M: 3/49, 1/50; F: 1/48, 0/49 Rats: M: 3/50, 2/50, 3/49; F: 0/50, 0/49, 2/50
Maltoni et al., 1986	S-D rats (M, F)	Gavage, 1/d, 4−5 d/wk, 56 wk; hold until death	0, 50 or 250 mg/kg	M: 0/30, 0/30, 0/30; F: 0/30, 0/30, 0/30	M: 0/30, 0/30, 0/30; F: 0/30, 0/30, 0/30

*Statistically-significantly different from controls by Fisher's exact test ($p < 0.05$).
**Statistically-significantly different from controls by Fisher's exact test ($p < 0.01$).

The inhalation studies by Fukuda et al. (1983), which involved female ICR mice and Sprague-Dawley rats, observed a 3-fold increase in lung tumors per mouse in those exposed to the two higher concentrations (150−450 ppm) but reported no increase in lung tumors in the rats. Maltoni et al. (1986, 1988) reported statistically-significantly increased pulmonary tumors in male Swiss and female B6C3F1 mice at the highest dose of 600 ppm, but no significant increases in any of the other species/strains/sexes tested. Henschler et al. (1980) tested NMRI mice, Wistar rats and Syrian hamsters of both sexes, and reported no observed increase in pulmonary tumors any of the species tested (see Section 4.4 and Appendix E for details of the conduct of these studies).

4.7.2.2.2. ***Gavage.*** None of the six chronic gavage studies (Van Duuren et al., 1979; NCI, 1976; Henschler et al., 1984; NTP, 1988, 1990; Maltoni et al., 1986), which exposed multiple strains of rats and mice to 0−3,000 mg/kg TCE for at least 56 weeks, reported a statistically-significant excess in lung tumors, although nonstatistically-significant increases were frequently observed in mice.

The study by Van Duuren et al. (1979) examined TCE along with 14 other halogenated compounds for carcinogenicity in both sexes of Swiss mice. While no excess tumors were observed, the dose rate of 0.5 mg once per week is equivalent to an average dose rate of approximately 2.4 mg/kg/d for a mouse weighing 30 g, which is about 400-fold smaller than that in the other gavage studies. In the NCI (1976) study, the results for Osborne-Mendel rats were considered inconclusive due to significant early mortality, but female B6C3F1 mice (though not males) exhibited a nonstatistically-significant elevation in pulmonary tumor incidence. The NCI study (1976) used technical grade TCE which contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane), but a later study by Henschler et al. (1984) in which mice were given TCE that was either pure, industrial, and stabilized with one or both of these stabilizers found similar pulmonary tumors regardless of the presence of stabilizers. In this study, female mice (n = 50) had elevated, but again not statistically-significant, increases in pulmonary tumors. A later gavage study by NTP (1988), which used TCE stabilized with diisopropylamine, observed no pulmonary tumors, but chemical toxicity and early mortality rendered this study inadequate for determining carcinogenicity. The final NTP study (1990) in male and female F344 rats and B6C3F1 mice, using epichlorohydrin-free TCE, again showed early mortality in male rats. Similar to the other gavage studies, a nonstatistically significant elevation in (malignant) pulmonary tumors was observed in mice, in this case in both sexes. These animal studies show that while there is a limited increase in lung tumors following gavage exposure to TCE in mice, the only statistically significant increase in lung tumors occurs following inhalation exposure in mice.

4.7.3. Role of Metabolism in Pulmonary Toxicity

TCE oxidative metabolism has been demonstrated to play a main role in TCE pulmonary toxicity in mice. However, data are not available on the role of specific oxidative metabolites in the lung. The Clara cell is thought to be the cell type responsible for much of the CYP metabolism in the lung. Therefore, damage to this cell type would be expected to also affect metabolism. More direct measures of CYP and isozyme-specific depression following TCE exposure have been reported following intraperitoneal administration in mice. Forkert et al. (1985) reported significant reduction in microsomal aryl hydrocarbon hydroxylase activity as well as CYP content between 1 and 24 hours after exposure (2,000−3,000 mg/kg i.p. TCE). Maximal depression occurred between 2 and 12 hours, with aryl hydrocarbon hydroxylase activity (a function of CYP) less than 50% of controls and CYP content less than 20% of controls. While there was a trend towards recovery from 12 to 24 hours, depression was still significant at 24 hours. Forkert et al. (2005) reported decreases in immunoreactive CYP2E1, CYP2F2, and CYP2B1 in the 4 hours after TCE treatment with 750 mg/kg intraperitoneal injection in mice. The amount and time of maximal reduction was isozyme dependent (CYP2E1: 30% of controls at 2 hours; CYP2F2: abolished at 30 minutes; CYP2B1: 43% of controls at 4 hours). Catalytic markers for CYP2E1, CYP2F2, and CYP2B enzymes showed rapid onset (15 minutes or less after TCE administration) of decreased activity, and continued depression through 4 hours. Decrease in CYP2E1 and CYP2F2 activity (measured by PNP hydroxylase activity) was greater than that of CYP2B (measured by pentoxyresorufin *O*-dealkylase activity). Forkert et al. (2006) reported similar results in which 4 hours after treatment, immunodetectable CYP2E1 protein was virtually abolished at doses 250−1,000 mg/kg and immunodetectable CYP2F2 protein, while still detectable, was reduced. PNP hydroxylase activity was also reduced 4 hours after treatment to 37% of controls at the lowest dose tested of 50 mg/kg, with further decreases to around 8% of control levels at doses of 500 mg/kg and higher. These results correlate with previously described increases in Clara cell cytotoxicity, as well as dichloroacetyl lysine (DAL) protein adduct formation. DAL adducts were observed in the bronchiolar epithelium of CD-1 mice and most prominent in the cellular apices of Clara cells (Forkert et al., 2006). This study also examined the effect of TCE *in vitro* exposure on the formation of chloral hydrate in lung microsomes from male CD-1 mice and CYP2E1 knock-out mice. The rates of CH formation were the same for lysosomes from both CD-1 and CYP2E1 knockout mice from 0.25 mM to 0.75 mM, but the CH formation peaked earlier for in the wild-type lysosomes (0.75 mM) as compared to CYP2E1-null lysosomes (1 mM).

The strongest evidence for the necessary role of TCE oxidation is that pretreatment of mice with diallyl sulfone ($DASO_2$), an inhibitor of CYP2E1 and CYP2F2, protected against TCE-induced pulmonary toxicity. In particular, following an intraperitoneal TCE dose of

750 mg/kg, Clara cells and the bronchiolar epithelium in mice pretreated with the CYP2E1/CYP2F2 inhibitor appeared normal. In naive mice given the same dose, the epithelium was attenuated due to exfoliation and there was clear morphological distortion of Clara cells (Forkert et al., 2005). In addition, the greater susceptibility of mouse lungs relative to rat lungs is consistent with their larger capacity to oxidize TCE, as measured *in vitro* in lung microsomal preparations (Green et al., 1997). Analysis by immunolocalization also found considerably higher levels of CYP2E1 in the mouse lung, heavily localized in Clara cells, as compared to rat lungs, with no detectable CYP2E1 in human lung samples (Green et al., 1997). In addition, both Green et al. (1997) and Forkert et al. (2006) report substantially lower metabolism of TCE in human lung microsomal preparations than either rats or mice. It is clear that CYP2E1 is not the only CYP enzyme involved in pulmonary metabolism, as lung microsomes from CYP2E1-null mice showed greater or similar rates of CH formation compared to those from wild-type mice. Recent studies have suggested a role for CYP2F2 in TCE oxidative metabolism, although more work is needed to make definitive conclusions. In addition, there may be substantial variability in human lung oxidative metabolism, as Forkert et al. (2006) reported that in microsomal samples from eight individuals, five exhibited no detectable TCE oxidation (<0.05 pmol/mg protein/20 minutes), while others exhibited levels well above the limit of detection (0.4−0.6 pmol/mg protein/minute).

In terms of direct pulmonary effects of TCE metabolites, Odum et al. (1992) reported that mice exposed to 100 ppm via inhalation of chloral for 6 hours resulted in bronchiolar lesions similar to those seen with TCE, although with a severity equivalent to 1,000 ppm TCE exposures. In addition, some alveolar necrosis, alveolar oedema, and desquamation of the epithelium were evident. In the same study, TCOH (100 and 500 ppm) also produced Clara cell damage, but with lower incidence than TCE, and without alveolar lesions, while TCA treatment produced no observable pulmonary effects. Therefore, it has been proposed that chloral is the active metabolite responsible for TCE pulmonary toxicity, and the localization of damage to Clara cells (rather than to other cell types, as seen with direct exposure to chloral) is due to the localization of oxidative metabolism in that cell type (Odum et al., 1992; Green et al., 1997; Green, 2000). However, the recent identification by Forkert et al. (2006) of DAL adducts, also localized with Clara cell, suggests that TCE oxidation to dichloroacetyl chloride, which is not believed to be derived from chloral, may also contribute to adverse health effects.

Due to the histological similarities between TCE- and chloral-induced pulmonary toxicity, consistent with chloral being the active moiety, it has been proposed that the limited or absent capacity for reduction of chloral (rapidly converted to CH in the presence of water) to TCOH and glucuronidation of TCOH to TCOG in mouse lungs leads to "accumulation" of chloral in Clara cells. However, the lack of TCOH glucuronidation capacity of Clara cells

reported by Odum et al. (1992), while possibly an important determinant of TCOH concentrations, should have no bearing on CH concentrations, which depend on the production and clearance of CH only. While isolated mouse Clara cells form smaller amounts of TCOH relative to CH (Odum et al., 1992), the cell-type distribution of the enzymes metabolizing CH is not clear. Indeed, cytosolic fractions of mouse, rat and human whole lungs show significant activity for CH conversion to TCOH (Green et al., 1997). In particular, in mouse lung subcellular fractions, 1 micromole of TCE in a 1.3 mL reactivial was converted to CH at a rate of 1 nmol/minute/mg microsomal protein, while 10 nmol CH in a 1.3 mL reactivial was converted to TCOH at a rate of 0.24 nmol/minute/mg cytosolic protein (Green et al., 1997). How this 4-fold difference in activity would translate *in vivo* is uncertain given the 100-fold difference in substrate concentrations, lack of information as to the concentration-dependence of activity, and uncertain differences between cytosolic and microsomal protein content in the lung. It is unclear whether local pulmonary metabolism of chloral is the primary clearance process *in vivo*, as in the presence of water, chloral rapidly converts to chloral hydrate, which is soluble in water and hence can rapidly diffuse to surrounding tissue and to the blood, which also has the capacity to metabolize chloral hydrate (Lipscomb et al., 1996). Nonetheless, experiments with isolated perfused lungs of rats and guinea pigs found rapid appearance of TCOH in blood following TCE inhalation exposure, with no detectable chloral hydrate or TCOG (Dalbey and Bingham, 1978). Therefore, it appears likely that chloral in the lung either is rapidly metabolized to TCOH, which then diffuses to blood, or diffuses to blood as CH and is rapidly metabolized to TCOH by erythrocytes (Lipscomb et al., 1996).

This hypothesis is further supported by *in vivo* data. No *in vivo* data in rats on CH after TCE administration were located, and Fisher et al. (1998) reported CH in blood of human volunteers exposed to TCE via inhalation were below detection limits. In mice, however, after both inhalation and oral gavage exposure to TCE, CH has been reported in whole lung tissue at concentrations similar to or somewhat greater than that in blood (Abbas and Fisher, 1997; Greenberg et al., 1999). A peak concentration (1.3 μg/g) of pulmonary CH was reported after inhalation exposure to 600 ppm—at or above exposures where Clara cell toxicity was reported in acute studies (Odum et al., 1992; Green et al., 1997). However, this was 5-fold *less* than the reported pulmonary CH concentration (6.65 μg/g) after gavage exposures of 1,200 mg/kg. Specifically, a 600-ppm exposure or 450-ppm exposure reported in the Maltoni et al. and Fukuda et al. studies results in a greater incidence in lung tumors than the 1,000−1,200 mg/kg/d exposures in the NTP (1990) and NCI (1976) bioassays. However, the peak CH levels measured in whole lung tissues after inhalation exposure to TCE at 600 ppm were reported to be about 5-fold *lower* than that at 1,200 mg/kg by gavage, therefore, showing the *opposite* pattern (Greenberg et al., 1999; Abbas and Fisher, 1997). No studies of Clara cell toxicity after gavage

exposures were located, but several studies in mice administered TCE via intraperitoneal injection did show Clara cell toxicity at around a dose of 750 mg/kg (Forkert et al., 2006) or above (e.g., Forkert and Forkert, 1994; Forkert and Birch, 1989). However, as noted previously, i.p. exposures are subject to an inflammatory response, confounding direct comparisons of dose via other routes of administration.

Although, whole lung CH concentrations may not precisely reflect the concentrations within specific cell types, as discussed above, the water solubility of CH suggests rapid equilibrium between cell types and between tissues and blood. Both Abbas and Fisher (1997) and Greenberg et al. (1999) were able to fit CH blood and lung levels using a PBPK model that did not include pulmonary metabolism, suggesting that lung CH levels may be derived largely by systemic delivery, i.e., from CH formed in the liver. However, a more detailed PBPK model-based analysis of this hypothesis has not been performed, as CH is not included in the PBPK model developed by Hack et al. (2006) that was updated in Section 3.5.

Two studies have reported formation of reactive metabolites in pulmonary tissues as assessed by macromolecular binding after TCE intraperitoneal administration. Forkert and Birch (1989) reported temporal correlations between the severity of Clara cell necrosis with increased levels of covalent binding macromolecules in the lung of TCE or metabolites with a single 2,000 mg/kg dose of [^{14}C] TCE. The amount of bound TCE or metabolites per gram of lung tissue, DNA, or protein peaked at 4 hours and decreased progressively at 8, 12, and 24 hours. The fraction of radioactivity in lung tissue macromolecules that was covalently bound reached a plateau of about 20% from 4−24 hours, suggesting that clearance of total and covalently bound TCE or metabolites was similar. The amount of covalent binding in the liver was 3- to 10-fold higher than in the lung, although hepatic cytotoxicity was not apparent. This tissue difference could either be due to greater localization of metabolism in the lung, so that concentrations reactive metabolites in individual Clara cells are greater than both the lung as a whole and hepatocytes, or because of greater sensitivity of Clara cells as compared to hepatocytes to reactive metabolites. More recently, Forkert et al. (2006) examined DAL adducts resulting from metabolism of TCE to dichloroacetyl chloride as an *in vivo* marker of production of reactive metabolites. Following intraperitoneal administration of 500−1,000 mg/kg TCE in CD-1 mice, they found localization of DAL adducts believed to be from oxidative metabolism within Clara cell apices, with dose-dependent increase in labeling with a polyclonal anti-DAL antibody that correlated with increased Clara cell damage. Dose-dependent DAL adducts were also found in alveolar Type II cells, although no morphologic changes in those cells were observed Both Clara cell damage (as discussed above) and DAL labeling were abolished in mice pretreated with $DASO_2$, an inhibitor of CYP2E1 and CYP2F2. However, Clara cell damage in treated CYP2E1-null mice was more severe than in CD-1 mice. Although DAL labeling was less pronounced in

CYP2E1-null mice as compared to CD-1 mice, this was due in part to the greater histopathologic damage leading to attenuation of the epithelium and loss of Clara cells in the null mice. In addition, protein immunoblotting with anti-DAL, anti-CYP2E1 and anti-CYP2F2 antibodies suggested that a reactive TCE metabolite including dichloroacetyl chloride was formed that is capable of binding to CYP2E1 and CYP2F2 and changing their protein structures. Follow-up studies are needed in the lung and other target tissues to determine the potential role of the DAL adducts in TCE-induced toxicity.

Finally, although Green (2000) and others have attributed species differences in pulmonary toxicity to differences in the capacity for oxidative metabolism in the lung, it should be noted that the concentration of the active metabolite is determined by both its production and clearance (Clewell et al., 2000). Therefore, while the maximal pulmonary capacity to produce oxidative metabolites is clearly greater in the mouse than in rats or humans, there is little quantitative information as to species differences in clearance, whether by local chemical transformation/metabolism or by diffusion to blood and subsequent systemic clearance. In addition, existing *in vitro* data on pulmonary metabolism are at millimolar TCE concentrations where metabolism is likely to be approaching saturation, so the relative species differences at lower doses has not been characterized. Studies with recombinant CYP enzymes examined species differences in the catalytic efficiencies of CYP2E1, CYP2F, and CYP2B1, but the relative contributions of each isoform to pulmonary oxidation of TCE *in vivo* remains unknown (Forkert et al., 2005). Furthermore, systemic delivery of oxidative metabolites to the lung may contribute, as evidenced by respiratory toxicity reported with i.p. administration. Therefore, while the differences between mice and rats in metabolic capacity are correlated with their pulmonary sensitivity, it is not clear that differences in capacity alone are accurate quantitative predictors of toxic potency. Thus, while it is likely that the human lung is exposed to lower concentrations of oxidative metabolites, quantitative estimates for differential sensitivity made with currently available data and dosimetry models are highly uncertain.

In summary, it appears likely that pulmonary toxicity is dependent on *in situ* oxidative metabolism, however, the active agent has not been confidently identified. The similarities in histopathologic changes in Clara cells between TCE and chloral inhalation exposure, combined with the wider range of cell types affected by direct chloral administration relative to TCE, led some to hypothesize that chloral is the toxic moiety in both cases, but with that generated *in situ* from TCE in Clara cells "accumulating" in those cells (Green, 2000). However, chemical and toxicokinetic data suggest that such "accumulation" is unlikely for several reasons. These include the rapid conversion of chloral to chloral hydrate in the presence of water, the water solubility of CH leading to rapid diffusion to other cell types and blood, the likely rapid metabolism of chloral hydrate to TCOH either in pulmonary tissue or in blood erythrocytes, and

in vivo data showing lack of correlation across routes of exposure between whole-lung CH concentrations and pulmonary carcinogenicity and toxicity. However, additional possibilities for the active moiety exist, such as dichloroacetyl chloride, which is derived through a TCE oxidation pathway independent of chloral and which appears to result in adducts with lysine localized in Clara cells.

4.7.4. Mode of Action for Pulmonary Carcinogenicity

A number of effects have been hypothesized to be key events in the pulmonary carcinogenicity of TCE, including cytotoxicity leading to increased cell proliferation, formation of DAL protein adducts, and mutagenicity. As stated previously, the target cell for pulmonary adenocarcinoma formation has not been established. Much of the hazard and MOA information has focused on Clara cell effects from TCE which is a target in both susceptible and nonsusceptible rodent species for lung tumors. However, the role of Clara cell susceptibility to TCE-induced lung toxicity or to other potential targets such as lung stem cells that are activated to repopulate both Clara and Type II alveolar cells after injury, has not been determined for pulmonary carcinogenicity. While all of the events described above may be plausibly involved in the MOA for TCE pulmonary carcinogenicity, none have been directly shown to be necessary for carcinogenesis.

4.7.4.1. *Mutagenicity via Oxidative Metabolism*

The hypothesis is that TCE acts by a mutagenic MOA in TCE- induced lung tumors. According to this hypothesis, the key events leading to TCE-induced lung tumor formation constitute the following: the oxidative metabolism of TCE producing chloral/chloral hydrate delivered to pulmonary tissues, causes direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

4.7.4.1.1. ***Experimental support for the hypothesized mode of action.*** Pulmonary toxicity has been proposed to be dependent on in situ oxidative metabolism, however, the active agent has not been confidently identified. The similarities in histopathologic changes in Clara cells between TCE and chloral inhalation exposure, combined with the wider range of cell types affected by direct chloral administration relative to TCE, led some to hypothesize that chloral is the toxic moiety. Chloral that is formed from the metabolism of TCE is quickly converted to CH upon hydration under physiological conditions. As discussed in Section 4.2.4, CH clearly induces aneuploidy in multiple test systems, including bacterial and fungal assays *in vitro* (Kafer, 1986; Kappas, 1989; Crebelli et al., 1991), mammalian cells *in vitro* (Vagnarelli et al., 1990; Sbrana et al., 1993), and mammalian germ-line cells *in vivo* (Russo et al., 1984; Miller and

Adler, 1992). Conflicting results were observed in *in vitro* and *in vivo* mammalian studies of micronuclei formation (Degrassi and Tanzarella, 1988; Nesslany and Marzin, 1999; Russo and Levis, 1992a, b; Giller et al., 1995; Beland, 1999), with positive results in germ-line cells (Nutley et al., 1996; Allen et al., 1994). In addition, it is mutagenic in the Ames bacterial mutation assay for some strains (Haworth et al., 1983; Ni et al., 1994; Beland, 1999; Giller et al., 1995). Structurally related chlorinated aldehydes 2-chloroacetyaldehyde and 2,2-dichloroacetaldehyde are both alkylating agents, are both positive in a genotoxic assay (Bignami et al., 1980), and both interact covalently with cellular macromolecules (Guengerich et al., 1979).

As discussed in the section describing the experimental support for the mutagenic MOA for liver carcinogenesis (see Section 4.5.7.1), it has been argued that CH mutagenicity is unlikely to be the cause of TCE carcinogenicity because the concentrations required to elicit these responses are several orders of magnitude higher that achieved *in vivo* (Moore and Harrington-Brock, 2000). Similar to the case of the liver, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays *in vitro* and concentrations *in vivo*, as reported *in vivo* CH concentrations are in whole lung homogenate while *in vitro* concentrations are in culture media. None of the available *in vivo* genotoxicity assays used the inhalation route that elicited the greatest lung tumor response under chronic exposure conditions, so direct *in vivo* comparisons are not possible. Finally, as discussed in Section 4.5.7.1, the use of i.p. administration in many other *in vivo* genotoxicity assays complicates the comparison with carcinogenicity data.

As discussed above (see Section 4.7.3), chemical and toxicokinetic data are not supportive of CH being the active agent of TCE-induced pulmonary toxicity, and directly contradict the hypothesis of chloral "accumulation." Nonetheless, CH has been measured in the mouse lung following inhalation and gavage exposures to TCE (Abbas and Fisher, 1997; Greenberg et al., 1999), possibly the result of both *in situ* production and systemic delivery. Therefore, in principle, CH could cause direct alterations in DNA in pulmonary tissue. However, as discussed above, the relative amounts of CH measured in whole lung tissue from inhalation and oral exposures do not appear to correlate with sensitivity to TCE lung tumor induction across exposure routes. While these data cannot rule out a role for mutagenicity mediated by CH due to various uncertainties, such as whether whole lung CH concentrations accurately reflect cell-type specific concentrations and possible confounding due to strain differences between inhalation and oral chronic bioassays, they do not provide support for this MOA.

Additional possibilities for the active moiety exist, such as dichloroacetyl chloride, which is derived through a TCE oxidation pathway independent of chloral and which appears to result

in adducts with lysine localized in Clara cells (Forkert et al., 2006). DCA, which has some genotoxic activity, is, also, presumed to be formed through this pathway (see Section 3.3). Currently, however, there are insufficient data to support a role for these oxidative metabolites in a mutagenic MOA.

4.7.4.2. *Cytotoxicity Leading to Increased Cell Proliferation*

The hypothesis is that TCE acts by a cytotoxicity MOA in TCE-induced pulmonary carcinogenesis. According to this hypothesis, the key events leading to TCE-induced lung tumor formation constitute the following: TCE oxidative metabolism *in situ* leads to currently unknown reactive metabolites that cause cytotoxicity, leading to compensatory cellular proliferation and subsequently increased mutations and clonal expansion of initiated cells.

4.7.4.2.1. ***Experimental support for the hypothesized mode of action.*** Evidence for the hypothesized MOA consists primarily of (1) the demonstration of acute cytotoxicity and transient cell proliferation following TCE exposure in laboratory mouse studies; (2) toxicokinetic data supporting oxidative metabolism being necessary for TCE pulmonary toxicity; (3) the association of lower pulmonary oxidative metabolism and lower potency for TCE-induced cytotoxicity with the lack of observed pulmonary carcinogenicity in laboratory rats. However, there is a lack of experimental support linking TCE acute pulmonary cytotoxicity to sustained cellular proliferation of chronic exposures or clonal expansion of initiated cells.

As discussed above, a number of acute studies have shown that TCE is particularly cytotoxic to Clara cells in mice, which has been suggested to be involved in the development of mouse lung tumors (Buckpitt et al., 1995; Forkert and Forkert, 1994, Kim et al., 2005). In addition, studies examining cell labeling by either BrdU (Green et al., 1997) or 3H-thymidine incorporation (Villaschi et al., 1991) suggest increased cellular proliferation in mouse Clara cells following acute inhalation exposures to TCE. Moreover, in short-term studies, Clara cells appear to become resistant to cytotoxicity with repeated exposure, but regain their susceptibility after 2 days without exposure. This observation led to the hypothesis that the 5 day/week inhalation dosing regime (Fukuda et al., 1983; Maltoni et al., 1986, 1988; Henschler et al., 1980) in the chronic mouse studies leads to periodic cytotoxicity in the mouse lung at the beginning of each week followed by cellular regeneration, and that the increased rate of cell division leads to increased incidence of tumors by increasing the overall mutation rate and by increasing the division rate of already initiated cells (Green, 2000). However, longer-term studies to test this hypothesis have not been carried out.

As discussed above (see Section 4.7.3), there is substantial evidence that pulmonary oxidative metabolism is necessary for TCE-induced pulmonary toxicity, although the active

moiety remains unknown. In addition, the lower capacity for pulmonary oxidative metabolism in rats as compared to mice is consistent with studies in rats not reporting pulmonary cytoxicity until exposures higher than those in the bioassays, and the lack of reported pulmonary carcinogenicity in rats at similar doses to mice. However, rats also have a lower background rate of lung tumors (Green, 2000), and so would be less sensitive to carcinogenic effects in that tissue to the extent that relative risks is the important metric across species. In addition, this MOA hypothesis requires a number of additional key assumptions for which there are currently no direct evidence. First, the cycle of cytotoxicity, repair, resistance to toxicity, and loss of resistance after exposure interruption, has not been documented and under the proposed MOA should continue under chronic exposure conditions. This cycle has thus, far only been observed in short term (up to 13-day) studies. In addition, although Clara cells have been identified as the target of toxicity whether they or endogenous stem cells in the lung are the cells responsible for mouse lung tumors has not been established. There is currently no data as to the cell type of origin for TCE-induced lung tumors.

4.7.4.3. *Additional Hypothesized Modes of Action with Limited Evidence or Inadequate Experimental Support*

4.7.4.3.1. ***Role of formation of DAL protein adducts.*** As discussed above, Forkert et al. (2006) recently observed dose-dependent formation of DAL protein adducts in the Clara cells of mice exposed to TCE via intraperitoneal injection. While adducts were highly localized in Clara cells, they were also found in alveolar Type II cells, though these cells did not show signs of cytotoxicity in this particular experimental paradigm. In terms of the MOA for TCE-induced pulmonary carcinogenicity, these adducts may either be causally important in and of themselves, or they may be markers of a different causal effect. For instance, it is possible that these adducts are a cause for the observed Clara cell toxicity, and Forkert et al. (2006) suggested that the lack of toxicity in alveolar Type II cells may indicate that "there may be a threshold in adduct formation and hence bioactivation at which toxicity is manifested." In this case, they are an additional precursor event in the same causal pathway proposed above. Alternatively, these adducts may be indicative of effects related to carcinogenesis but unrelated to cytotoxicity. In this case, the Clara cell need not be the cell type of origin for mouse lung tumors.

Because of their recent discovery, there is little additional data supporting, refuting, or clarifying the potential role for DAL protein adducts in the MOA for TCE-induced pulmonary carcinogenesis. For instance, the presence and localization of such adducts in rats has not been investigated, and could indicate the extent to which the level of adduct formation is correlated with existing data on species differences in metabolism, cytotoxicity, and carcinogenicity. In addition, the formation of these adducts has only been investigated in a single dose study using

i.p. injection. As stated above, i.p. injection may involve the initiation of a systemic inflammatory response that can activate lung macrophages or affect Clara cells. Experiments with repeated exposures over chronic durations and by inhalation or oral of administration would be highly informative. Finally, the biological effects of these adducts, whether cytotoxicity or something else, have not been investigated.

4.7.4.4. *Conclusions About the Hypothesized Modes of Action*

4.7.4.4.1. *(1) Is the hypothesized mode of action sufficiently supported in the test animals?*

4.7.4.4.1.1. *Mutagenicity*. Chloral hydrate is clearly genotoxic, as there are substantial data from multiple *in vitro* and *in vivo* assays supporting its ability induce aneuploidy, with more limited data as to other genotoxic effects, such as point mutations. Chloral hydrate is also clearly present in pulmonary tissues of mice following TCE exposures similar to those inducing lung tumors in chronic bioassays. However, chemical and toxicokinetic data are not supportive of CH being the predominant metabolite for TCE carcinogenicity. Such data include the water solubility of CH leading to rapid diffusion to other cell types and blood, its likely rapid metabolism to TCOH either in pulmonary tissue or in blood erythrocytes, and *in vivo* data showing lack of correlation across routes of exposure between whole lung CH concentrations and pulmonary carcinogenicity. Therefore, while a role for mutagenicity via CH in the MOA of TCE-induced lung tumors cannot be ruled about, available evidence is inadequate to support the conclusion that direct alterations in DNA caused by CH produced in or delivered to the lung after TCE exposure constitute a MOA for TCE-induced lung tumors.

4.7.4.4.1.2. *Cytotoxicity*. The MOA hypothesis for TCE-induced lung tumors involving cytotoxicity is supported by relatively consistent and specific evidence for cytotoxicity at tumorigenic doses in mice. However, the majority of cytotoxicity-related key events have been investigated in studies less than 13 days, and none has been shown to be causally related to TCE-induced lung tumors. In addition, the cell type (or types) of origin for the observed lung tumors in mice has not been determined, so the contribution to carcinogenicity of Clara cell toxicity and subsequent regenerative cell division is not known. Similarly, the relative contribution from recently discovered dichloroacetyl-lysine protein adducts to the tumor response has not been investigated and has currently only been studied in i.p. exposure paradigms of short duration. In summary, while there are no data directly challenging the hypothesized MOA described above, the existing support for their playing a causal role in TCE-induced lung tumors is largely associative, and based on acute or short term studies. Therefore, there are inadequate data to support a cytotoxic MOA based on the TCE-induced cytotoxicity in Clara cells in the lungs of test animals.

4.7.4.4.1.3. ***Additional hypothesis.*** Inadequate data are available to develop a MOA hypothesis based on recently discovered DAL adducts induced by TCE inhalation and i.p. exposures. It will therefore, not be considered further in the conclusions below.

Overall, therefore, the MOA for TCE-induced lung tumors is considered unknown at this time.

4.7.4.4.2. ***(2) Is the hypothesized mode of action relevant to humans?***

4.7.4.4.2.1. ***Mutagenicity.*** The evidence discussed above demonstrates that CH is mutagenic in microbial as well as test animal species. There is therefore, the presumption that they would be mutagenic in humans. Therefore, this MOA is considered relevant to humans.

4.7.4.4.2.2. ***Cytotoxicity.*** No data from human studies are available on the cytotoxicity of TCE and its metabolites in the lung, and no causal link between cytotoxicity and pulmonary carcinogenicity has been demonstrated in animal or human studies. Nonetheless, in terms of human relevance, no data suggest that the proposed key events are not biologically plausible in humans, therefore, qualitatively, TCE-induced lung tumors are considered relevant to humans. Information about the relative pharmacodynamic sensitivity between rodents and humans is absent, but information on pharmacokinetic differences in lung oxidative metabolism does exist and will be considered in dose-response assessment when extrapolating between species (see Section 5.2.1.2).

4.7.4.4.3. ***(3) Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?***

4.7.4.4.3.1. ***Mutagenicity.*** The mutagenic MOA is considered relevant to all populations and lifestages. According to U.S. EPA's *Cancer Guidelines* (U.S. EPA, 2005a) and *Supplemental Guidance* (U.S. EPA, 2005b), there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic mode of action. However, because the weight of evidence is inadequate to support a mutagenic MOA for TCE pulmonary carcinogenicity, and in the absence of chemical-specific data to evaluate differences in susceptibility, the ADAFs should not be applied, in accordance with the *Supplemental Guidance.*

4.7.4.4.3.2. ***Cytotoxicity.*** No information based is available as to which populations or lifestages may be particularly susceptible to TCE-induced lung tumors. However, pharmacokinetic differences in lung oxidative metabolism among humans do exist, and because of the association between lung oxidative metabolism and toxicity, will be considered in dose-response assessment when extrapolating within species.

4.7.5. Summary and Conclusions

The studies described here show pulmonary toxicity found mainly in Clara cells in mice (Green et al., 1997; Villaschi et al., 1991; Odum et al., 1992; Forkert et al., 1985; Forkert and Birch, 1989) and rats (Kurasawa, 1988). The most convincing albeit limited data regarding this type of toxicity was demonstrated predominantly in mice exposed via inhalation, although some toxicity was shown in intraperitoneal injection studies. Increased vacuolation of Clara cells was often seen within the first 24-hours-of-exposure, depending on dose, but with cellular repair occurring within days or weeks of exposure. Continued exposure led to resistance to TCE-induced Clara cell toxicity, but damage recurred if exposure was stopped after 5 days and then resumed after 2 days without exposure. However, Clara cell toxicity has only been observed in acute and short-term studies, and it is unclear whether they persist with subchronic or chronic exposure, particularly in mice, which are the more sensitive species. With respect to pulmonary carcinogenicity, statistically-significantly increased incidence of lung tumors from chronic inhalation exposures to TCE was observed female ICR mice (Fukuda et al., 1983), male Swiss mice, and female B6C3F1 mice (Maltoni et al., 1986), though not in other sex/strain combinations, nor in rats (Henschler et al., 1980; Maltoni et al., 1986). However, lung toxicity and Clara cell effects have also been observed in rats. Overall, the limited carcinogenesis studies described above are consistent with TCE causing mild increases in pulmonary tumor incidence in mice, but not in other species tested such as rats and hamsters.

The epidemiologic studies are quite limited for examining the role of TCE in cancers of the respiratory system, with no studies found on TCE exposure specifically examining toxicity of the respiratory tract. The two studies found on organic solvent exposure which included TCE suggested smoking as a primary factor for observed lung function decreases among exposed workers. Animal studies have demonstrated toxicity in the respiratory tract, particularly damage to the Clara cells (nonciliated bronchial epithelial cells), as well as decreases in pulmonary surfactant following both inhalation and intraperitoneal exposures, especially in mice. Dose-related increases in vacuolation of Clara cells have been observed in mice and rats as early as 24 hours postexposure (Odum et al., 1992; Kurasawa, 1988; Forkert et al., 1985, 2006; Forkert and Birch, 1989; Scott et al., 1988). Mice appear to be more sensitive to these changes, but both species show a return to normal cellular morphology at four weeks postexposure (Odum et al., 1992). Studies in mice have also shown an adaptation or resistance to this damage after only 4 to 5 days of repeated exposures (Odum et al., 1992; Green et al., 1997). The limited epidemiological literature on lung and laryngeal cancer in TCE-exposed groups is inconclusive due to study limitations (low power, null associations, confidence intervals on relative risks that include 1.0). These studies can only rule out risks of a magnitude of 2.0 or greater for lung cancer and relative risks greater than 3.0 or 4.0 for laryngeal cancer for exposures to studied

populations and thus, may not detect a level of response consistent with other endpoints. Animal studies demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE (Fukuda et al., 1983; Maltoni et al., 1988, 1986). These results were not seen in other species tested (rats, hamsters; Maltoni et al., 1986, 1988; Fukuda et al., 1983; Henschler et al., 1980). By gavage, elevated, but not statistically significant, incidences of benign and/or malignant pulmonary tumors have been reported in B6C3F1 mice (NCI, 1976; Henschler et al., 1984; NTP, 1990). No increased pulmonary tumor incidences have been reported in rats exposed to TCE by gavage (NCI, 1976; NTP, 1988, 1990), although all the studies suffered from early mortality in at least one sex of rat.

Although no epidemiologic studies on the role of metabolism of TCE in adverse pulmonary health effects have been published, animal studies have demonstrated the importance of the oxidative metabolism of TCE by CYP2E1 and/or CYP2F2 in pulmonary toxicity. Exposure to diallyl sulfone ($DASO_2$), an inhibitor of both enzymes protects against pulmonary toxicity in mice following exposure to TCE (Forkert et al., 2005). The increased susceptibility in mice correlates with the greater capacity to oxidize TCE based on increased levels of CYP2E1 in mouse lungs relative to lungs of rats and humans (Green et al., 1997; Forkert et al., 2006), but it is not clear that these differences in capacity alone are accurate quantitative predictors of sensitivity to toxicity. In addition, available evidence argues against the previously proposed hypothesis (e.g., Green, 2000) that "accumulation" of chloral in Clara cells is responsible for pulmonary toxicity, since chloral is first converted the water-soluble compounds chloral hydrate and TCOH that can rapidly diffuse to surrounding tissue and blood. Furthermore, the observation of DAL protein adducts, likely derived dichloroacetyl chloride and not from chloral, that were localized in Clara cells suggests an alternative to chloral as the active moiety. While chloral hydrate has shown substantial genotoxic activity, chemical and toxicokinetic data on CH as well as the lack of correlation across routes of exposure between *in vivo* measurements of CH in lung tissues and reported pulmonary carcinogenicity suggest that evidence is inadequate to conclude that a mutagenic MOA mediated by CH is operative for TCE-induced lung tumors. Another MOA for TCE-induced lung tumors has been plausibly hypothesized to involve cytotoxicity leading to increased cell proliferation, but the available evidence is largely associative and based on short-term studies, so a determination of whether this MOA is operative cannot be made. The recently discovered formation of DAL protein adducts in pulmonary tissues may also play a role in the MOA of TCE-induced lung tumors, but an adequately defined hypothesis has yet to be developed. Therefore, the MOA for TCE-induced lung tumors is currently considered unknown, and this endpoint is thus, considered relevant to humans. Moreover, none of the available data suggest that any of the currently hypothesized mechanisms would be biologically precluded in humans.

4.8. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

4.8.1. Reproductive Toxicity

An assessment of the human and experimental animal data, taking into consideration the overall weight of the evidence, demonstrates a concordance of adverse reproductive outcomes associated with TCE exposures. Effects on male reproductive system integrity and function are particularly notable and are discussed below. Cancers of the reproductive system in both males and females have also been identified and are discussed below.

4.8.1.1. *Human Reproductive Outcome Data*

A number of human studies have been conducted that examined the effects of TCE on male and female reproduction following occupational and community exposures. These are described below and summarized in Table 4-74. Epidemiological studies of female human reproduction examined infertility and menstrual cycle disturbances related to TCE exposure. Other studies of exposure to pregnant women are discussed in the section on human developmental studies (see Section 4.8.2.1). Epidemiological studies of male human reproduction examined reproductive behavior, altered sperm morphology, altered endocrine function, and infertility related to TCE exposure.

4.8.1.1.1. *Female and male combined human reproductive effects.*

Reproductive behavior. A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the reproductive outcomes in 75 men and 71 women exposed to TCE in drinking water (ATSDR, 2001). TCE exposure was classified as high (>10.0 ppb), medium (≥5.0 to <10.0 ppb), and low (<5.0 ppb). Altered libido for men and women combined was observed in a dose-response fashion, although the results were nonsignificant. The results were not stratified by gender.

4.8.1.1.2. *Female human reproductive effects.*

4.8.1.1.2.1. ***Infertility.*** Sallmén et al. (1995) examined maternal occupational exposure to organic solvents and time-to-pregnancy. Cases of spontaneous abortion and controls from a prior study of maternal occupational exposure to organic solvents in Finland during 1973−1983 and pregnancy outcome (Lindbohm et al., 1990) were used to study time-to-pregnancy of 197 couples. Exposure was assessed by questionnaire during the first trimester and confirmed with employment records. Biological measurements of TCA in urine in 64 women who held the same job during pregnancy and measurement (time of measurement not stated) had a median value of 48.1 µmol/L (mean: 96.2 ± 19.2 µmol/L) (Lindbohm et al., 1990). Nineteen women had low exposure to TCE (used <1 or 1−4 times/week), and 9 had high exposure to TCE (daily use).

In this follow-up study, an additional questionnaire on time-to-pregnancy was answered by the mothers (Sallmén et al., 1995). The incidence density ratio (IDR) was used in this study to estimate the ratio of average incidence rate of pregnancies for exposed women compared to nonexposed women; therefore, a lower IDR indicates infertility. For TCE, a reduced incidence of fecundability was observed in the high exposure group (IDR: 0.61, 95% CI: 0.28−1.33) but not in the low exposure group (IDR: 1.21, 95% CI: 0.73−2.00). A similar study of paternal occupational exposure (Sallmén et al., 1998) is discussed in Section 4.2.1.2.

The residential study in Colorado discussed above did not observe an effect on lifetime infertility infertility in the medium (OR_{adj}: 0.45; 95% CI: 0.02−8.92) or high exposure groups (OR_{adj}: 0.88; 95% CI: 0.13−6.22) (ATSDR, 2001). Curiously, exposed women had more pregnancies and live births than controls.

4.8.1.1.2.2. ***Menstrual cycle disturbance.*** The ATSDR (2001) study discussed above also examined effects on the menstrual cycle (ATSDR, 2001). Nonsignificant associations without a dose-response were seen for abnormal menstrual cycle in women (OR_{adj}: 2.23, 95% CI: 0.45−11.18).

Other studies have examined the effect of TCE exposure on the menstrual cycle. One study examined women working in a factory assembling small electrical parts (Zielinski, 1973, translated). The mean concentration of TCE in indoor air was reported to be 200 mg/m^3. Eighteen percent of the 140 exposed women suffered from amenorrhea, compared to only 2% of the 44 nonexposed workers. The other study examined 75 men and women working in dry cleaning or metal degreasing (Bardodej and Vyskocil, 1956). Exposures ranged from 0.28−3.4 mg/L, and length of exposure ranged from 0.5 to 25 years. This study reported that many women experienced menstrual cycle disturbances, with a trend for increasing air concentrations and increasing duration of exposure.

An additional case study of a 20-year-old woman was occupationally exposed to TCE via inhalation. The exposure was estimated to be as high as 10 mg/mL or several thousand ppm, based on urine samples 21−25 days after exposure of 3.2 ng/mL of total trichloro-compounds. The primary effect was neurological, although she also experienced amenorrhea, followed by irregular menstruation and lack of ovulation as measured by basal body temperature curves (Sagawa et al., 1973).

4.8.1.1.3. ***Male human reproductive effects.***

4.8.1.1.3.1. ***Reproductive behavior.*** One study reported on the effect of TCE exposure on the male reproductive behavior in 75 men working in dry cleaning or metal degreasing (Bardodej and Vyskocil, 1956). Exposures ranged from 0.28−3.4 mg/L, and length of exposure ranged

from 0.5 to 25 years. This study found that men experienced decreased potency or sexual disturbances; the authors speculated that the effects on men could be due to the CNS effects of TCE exposure. This study also measured serial neutral 17-ketosteroid determinations but they were found to be not statistically significant (Bardodej and Vyskocil, 1956).

An occupational study of 30 men working in a money printing shop were exposed to TCE for <1 year to 5 years (El Ghawabi et al., 1973). Depending on the job description, the exposures ranged from 38−172-ppm TCE. Ten (33%) men suffered from decreased libido, compared to three (10%) of unexposed controls. However, these results were not stratified by exposure level or duration. The authors speculate that decreased libido was likely due to the common symptoms of fatigue and sleepiness.

A case study described a 42 year-old man exposed to TCE who worked as an aircraft mechanic for approximately 25 years (Saihan et al., 1978). He suffered from a number of health complaints including gynaecomastia and impotence, along with neurotoxicity and immunotoxicity. In addition, he drank alcohol daily which could have increased his response to TCE.

4.8.1.1.3.2. ***Altered sperm quality.*** Genotoxic effects on male reproductive function were examined in a study evaluating occupational TCE exposure in 15 male metal degreasers (Rasmussen et al., 1988). No measurement of TCE exposure was reported. Sperm count, morphology, and spermatozoa Y-chromosomal nondisjunction during spermatogenesis were examined, along with chromosomal aberrations in cultured lymphocytes. A nonsignificant increase in percentage of two fluorescent Y-bodies (YFF) in spermatozoa were seen in the exposed group ($p > 0.10$), and no difference was seen in sperm count or morphology compared to controls.

An occupational study of men using TCE for electronics degreasing (Chia et al., 1996, 1997; Goh et al., 1998) examined subjects ($n = 85$) who were offered a free medical exam if they had no prior history related to endocrine function, no clinical abnormalities, and normal liver function tests; no controls were used. These participants provided urine, blood, and sperm samples. The mean urine TCA level was 22.4 mg/g creatinine (range: 0.8−136.4 mg/g creatinine). In addition, 12 participants provided personal 8-hour air samples, which resulted in a mean TCE exposure of 29.6 ppm (range: 9−131 ppm). Sperm samples were divided into two exposure groups; low for urine TCE less than 25 mg/g creatinine, and high for urine TCA greater than or equal to 25 mg/g creatinine. A decreased percentage of normal sperm morphology was observed in the sperm samples in the high exposure group ($n = 48$) compared to the low exposure group ($n = 37$). However, TCE exposure had no effect on semen volume, sperm

density, or motility. There was also an increased prevalence of hyperzoospermia (sperm density of >120 million sperm per mL ejaculate) with increasing urine TCA levels (Chia et al., 1996).

4.8.1.1.3.3. ***Altered endocrine function.*** Two studies followed up on the study by Chia et al. (1996) to examine endocrine function (Chia et al., 1997; Goh et al., 1998). The first examined serum testosterone, follicle-stimulating hormone (FSH), dehydroepiandrosterone sulphate (DHEAS), and sex-hormone binding globulin (SHBG) (Chia et al., 1997). With increased years of exposure to TCE, an increase in DHEAS levels were seen, from 255 ng/mL for <3 years to 717.8 ng/mL ≥7 years exposure. Also with increased years of exposure to TCE, decreased FSH, SHBG and testosterone levels were seen. The authors speculated these effects could be due to decreased liver function related to TCE exposure (Chia et al., 1997).

The second follow-up study of this cohort studied the hormonal effects of chronic low-dose TCE exposure in these men (Goh et al., 1998). Because urine TCE measures only indicate short-term exposure, long-term exposure was indicated by years of exposure. Hormone levels examined include androstenedione, cortisol, testosterone, aldosterone, SHBG, and insulin. Results show that a decrease in serum levels of testosterone and SHBG were significantly correlated with years of exposure to TCE, and an increase in insulin levels were seen in those exposed for less than 2 years. Androstenedione, cortisol, and aldosterone were in normal ranges and did not change with years of exposure to TCE.

4.8.1.1.3.4. ***Infertility.*** Sallmén et al. (1998) examined paternal occupational exposure and time-to-pregnancy among their wives. Cases of spontaneous abortion and controls from a prior study of pregnancy outcome (Taskinen et al., 1989) were used to study time-to-pregnancy of 282 couples. Exposure was determined by biological measurements of the father who held the same job during pregnancy and measurement (time of measurement not stated) and questionnaires answered by both the mother and father. An additional questionnaire on time-to-pregnancy was answered by the mother for this study six years after the original study (Sallmén et al., 1998). The level of exposure was determined by questionnaire and classified as "low/intermediate" if the chemical was used <1 or 1−4 days/week and biological measures indicated high exposure (defined as above the reference value for the general population), and "high" if used daily or if biological measures indicated high exposure. For 13 men highly exposed, mean levels of urine TCA were 45 µmol/L (SD 42 µmol/L; median 31 µmol/L); for 22 men low/intermediately exposed, mean levels of urine TCA were 41 µmol/L (SD 88 µmol/L; median 15 µmol/L). The terminology IDR was replaced by fecundability density ratio (FDR) in order to reflect that pregnancy is a desired outcome; therefore, a high FDR indicates infertility. No effect was seen on fertility in the low exposure group (FDR: 0.99, 95% CI: 0.63−1.56) or in

the intermediate/high exposure group (FDR: 1.03, 95% CI: 0.60−1.76). However, the exposure categories were grouped by low/intermediate versus high, whereas the outcome categories were grouped by low versus intermediate/high, making a dose-response association difficult.

A small occupational study reported on eight male mechanics exposed to TCE for at least two years who sought medical treatment for infertility (Forkert et al., 2003). The wives were determined to have normal fertility. Samples of urine from two of the eight male mechanics contained TCA and/or TCOH, demonstrating the rapid metabolism in the body. However, samples of seminal fluid taken from all eight individuals detected TCE and the metabolites chloral hydrate and TCOH, with two samples detecting DCA and one sample detecting TCA. Five unexposed controls also diagnosed with infertility did not have any TCE or metabolites in samples of seminal fluid. There was no control group that did not experience infertility. Increased levels of TCE and its metabolites in the seminal fluid of exposed workers compared to lower levels found in their urine samples was explained by cumulative exposure and mobilization of TCE from adipose tissue, particularly that surrounding the epididymis. In addition, CYP2E1 was detected in the epididymis, demonstrating that metabolism of TCE can occur in the male reproductive tract. However, this study could not directly link TCE to the infertility, as both the exposed and control populations were selected due to their infertility.

The ATSDR (2001) study discussed above on the reproductive effects from TCE in drinking water of individuals living near the Rocky Mountain Arsenal in Colorado did not observe infertility or other adverse reproductive effects for the high exposure group compared to the low exposure group (OR_{adj}: 0.83; 95% CI: 0.11−6.37). Curiously, exposed men had more pregnancies and live births than controls.

4.8.1.1.4. ***Summary of human reproductive toxicity.*** Following exposure to TCE, adverse effects on the female reproductive system observed include reduced incidence of fecundability (as measured by time-to-pregnancy) and menstrual cycle disturbances. Adverse effects on the male reproductive system observed include altered sperm morphology, hyperzoospermia, altered endocrine function, decreased sexual drive and function, and altered fertility. These are summarized in Table 4-74.

4.8.1.2. *Animal Reproductive Toxicity Studies*

A number of animal studies have been conducted that examined the effects of TCE on reproductive organs and function following either inhalation or oral exposures. These are described below and summarized in Tables 4-75 and 4-76. Other animal studies of offspring exposed during fetal development are discussed in the section on animal developmental studies (see Section 4.8.2.2).

Table 4-74. Human reproductive effects

Subjects	Exposure	Effect	Reference
Female and male combined effects			
Reproductive behavior			
75 men and 71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Medium: ≥5.0−<10.0 ppb High: <10.0 ppb Highest: <15 ppb	Altered libido [a] Low: referent Med: OR_{adj}: 0.67 (95% CI: 0.18−2.49) High: OR_{adj}: 1.65 (95% CI: 0.54−5.01) Highest: OR_{adj}: 2.46 (95% CI: 0.59−10.28)	ATSDR, 2001
Female effects			
Infertility			
197 women occupationally exposed to solvents in Finland 1973−1983	U-TCA (μmol/L) [b] Median: 48.1 Mean: 96.2 ± 19.2	Reduced incidence of fecundability in the high exposure group [c] as measured by time to pregnancy Low: IDR = 1.21 (95%CI: 0.73−2.00) High: IDR = 0.61 (95%CI: 0.28−1.33)	Sallmén et al., 1995
71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Med: ≥5.0 to <10.0 ppb High: <10.0 ppb	No effect on lifetime infertility [a] Low: referent Med: OR_{adj}: 0.45 (95% CI: 0.02−8.92) High: OR_{adj}: 0.88 (95% CI: 0.13−6.22)	ATSDR, 2001
Menstrual cycle disturbance			
71 women living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Med: ≥5.0 to <10.0 ppb High: <10.0 ppb	Increase in abnormal menstrual cycle (defined as <26 days or >30 days) Low: referent Med: OR_{adj}: 4.17 (95% CI: 0.31−56.65) High: OR_{adj}: 2.39 (95% CI: 0.41−13.97)	ATSDR, 2001
184 women working in a factory assembling small electrical parts in Poland	Mean indoor air TCE: 200 mg/m^3	18% reporting increase in amenorrhea in exposed group (n = 140), compared to 2% increase in unexposed group (n = 44)	Zielinski, 1973
32 women working in dry cleaning or metal degreasing in Czechoslovakia[d]	0.28−3.4 mg/L TCE for 0.5−25 yrs	31% reporting increase in menstrual disturbances [a]	Bardodej and Vyskocil, 1956
20-yr-old woman was occupationally exposed to TCE via inhalation	Urine total trichloro-compounds 3.2 ng/mL (21−25 days after exposure)	Amenorrhea, followed by irregular menstruation and lack of ovulation	Sagawa et al., 1973
Male effects			
Reproductive behavior			
43 men working in dry cleaning or metal degreasing in Czechoslovakia	0.28−3.4 mg/L TCE for 0.5−25 yrs	30% reporting decreased potency [a]	Bardodej and Vyskocil, 1956
30 male workers in a money printing shop in Egypt	38−172 ppm TCE	Decreased libido reported in 10 men (33%), compared to 3 men in the control group (10%)	El Ghawabi et al., 1973
42 yr-old male aircraft mechanic in UK	TCE exposure reported but not measured; exposure for 25 yrs	Gynaecomastia, impotence	Saihan et al., 1978

1

Table 4-74. Human reproductive effects (continued)

Subjects	Exposure	Effect	Reference
Altered sperm quality			
15 men working as metal degreasers in Denmark	TCE exposure reported but not measured	Nonsignificant increase in percentage of two YFF in spermatozoa; no effect on sperm count or morphology	Rasmussen et al., 1988
85 men of Chinese descent working in an electronics factory	Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Decreased normal sperm morphology and hyperzoospermia	Chia et al., 1996
Altered endocrine function			
85 men of Chinese descent working in an electronics factory	Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Increased DHEAS and decreased FSH, SHBG and testosterone levels; dose-response observed	Chia et al., 1997
85 men of Chinese descent working in an electronics factory	Mean personal air TCE: 29.6 ppm; Mean U-TCA: 22.4 mg/g creatinine	Decreased serum levels of testosterone and SHBG were significantly correlated with years of exposure to TCE; increased insulin levels for exposure <2 yrs	Goh et al., 1998
Infertility			
282 men occupationally exposed to solvents in Finland 1973−1983	U-TCA (µmol/L): High exposure: [c] Mean: 45 (SD 42) Median 31 Low exposure: [c] Mean: 41 (SD 88) Median: 15	No effect on fecundability [c] (as measured by time to pregnancy) Low: FDR: 0.99 (95% CI: 0.63−1.56) Intermediate/High: FDR:[c] 1.03 (95% CI: 0.60−1.76)	Sallmén et al., 1998
8 male mechanics seeking treatment for infertility in Canada	Urine (µmol/): TCA: <0.30−4.22 TCOH: <0.60−0.89 Seminal fluid (pg/extract): TCE: 20.4−5,419.0 Chloral: 61.2−1,739.0 TCOH 2.7−25.5 TCA: <100−5,504 DCA: <100−13,342	Infertility could not be associated with TCE as controls were 5 men also in treatment for infertility	Forkert et al., 2003
75 men living near Rocky Mountain Arsenal, Colorado	Low: <5.0 ppb Med: ≥5.0 to <10.0 ppb High: <10.0 ppb	No effect on lifetime infertility (not defined) Low: referent Med: n/a High: OR_{adj}: 0.83 (95% CI: 0.11−6.37)	ATSDR, 2001

2
3 [a]Not defined by the authors.
4 [b]As reported in Lindbohm et al. (1990).
5 [c]Low/intermediate exposure indicated use of TCE <1 or 1−4 days/week, and biological measures indicated high
6 exposure. High exposure indicated daily use of TCE, or if biological measures indicated high exposure.
7 [d]Number inferred from data provided in Tables 2 and 3 in Bardodej and Vyskocil (1956).
8
9 UK = United Kingdom.

Table 4-75. Summary of mammalian *in vivo* reproductive toxicity studies—inhalation exposures

1

Reference	Species/strain/ sex/number	Exposure level/duration	NOAEL; LOAEL [a]	Effects
Forkert et al., 2002	Mouse, CD-1, male, 6/group	0 or 1,000 ppm (5,374 mg/m^3)[b] 6 h/d, 5 d/wk, 19 d over 4 wks	LOAEL: 1,000 ppm	U-TCA and U-TCOH increased by 2nd and 3rd wk, respectively. Cytochrome P450 2E1 and *p*-nitrophenol hydroxylation in epididymal epithelium > testicular Leydig cells. Choral also generated from TCE in epididymis > testis. Sloughing of epididymal epithelial cells after 4 wk exposure.
Kan et al., 2007	Mouse, CD-1, male, 4/group	0 or 1,000 ppm 6 h/d,5 d/wk, 1 to 4 wks	LOAEL: 1,000 ppm	Light microscopy findings: degeneration and sloughing of epididymal epithelial cells as early as 1 wk into exposure; more severe by 4 wks. Ultrastructional findings: vesiculation in cytoplasm, disintegration of basolateral cell membranes, sloughing of epithelial cells. Sperm found *in situ* in cytoplasm of degenerated epididymal cells. Abnormalities of the head and tail in sperm located in the epididymal lumen.
Kumar et al., 2000a	Rat, Wistar, male, 12−13/group	0 or 376 ppm 4 h/d, 5 d/wk, 2 to 10 wks exposure, 2 to 8 wks rest period	LOAEL: 376 ppm	Alterations in testes histopathology (smaller, necrotic spermatogenic tubules), ↑ sperm abnormalities, and sig. ↑ pre- and/or postimplantation loss in litters observed in the groups with 2 or 10 wks of exposure, or 5 wks of exposure with 2 wks rest.
Kumar et al., 2000b	Rat, Wistar, males, 12−13/group	0 or 376 ppm 4 h/d, 5 d/wk, 12 and 24 wks	LOAEL: 376 ppm	Sig. ↓ in total epididymal sperm count and sperm motility, with sig. ↓ in serum testosterone, sig. ↑ in testes cholesterol, sig. ↓ of glucose 6-phosphate dehydrogenase and 17-β-hydroxy steroid dehydrogenase at 12 and 24 wks exposure.
Kumar et al., 2001	Rat, Wistar, male, 6/group	0 or 376 ppm 4 h/d, 5 d/wk, 12 and 24 wks	LOAEL: 376 ppm	BW gain sig. ↓. Testis weight, sperm count and motility sig. ↓, effect stronger with exposure time. After 12 wk, numbers of spermatogenic cells and spermatids ↓, some of the spermatogenic cells appeared necrotic. After 24 wk testes were atrophied, tubules were smaller, had Sertoli cells and were almost devoid of spermatocytes and spermatids. Leydig cells were hyperplastic. SDH, G6PDH sig. ↓, GGT and β-glucuronidase sig. ↑; effects stronger with exposure time.
Land et al., 1981	Mouse, C57BlxC3H (F1), male, 5 or 10/group	0, 0.02%, or 0.2% 4 h/d, 5 d, 23 d rest	NOAEL: 0.02% LOAEL: 0.2%	Sig. ↑ percent morphologically abnormal epididymal sperm.

Table 4-75. Summary of mammalian *in vivo* reproductive toxicity studies—inhalation exposures (continued)

Reference	Species/strain/ sex/number	Exposure level/duration	NOAEL; LOAEL [a]	Effects
Xu et al., 2004	Mouse, CD-1, male, 4 to 27/group	0 or 1,000 ppm (5.37 mg/L)[b] 6 h/d, 5 d/wk, 1−6 wks	LOAEL: 1,000 ppm	Sig. ↓ *in vitro* sperm-oocyte binding and *in vivo* fertilization

[a]NOAEL and LOAEL are based upon reported study findings.
[b]Dose conversion calculations by study author(s).

Table 4-76. Summary of mammalian *in vivo* reproductive toxicity studies—oral exposures

1

Reference	Species/strain/ sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL [a]	Effects
Studies assessing male reproductive outcomes					
DuTeaux et al., 2003	Rat, Sprague-Dawley, male, 3/group	0, 0.2, or 0.4% (0, 143, or 270 mg/kg/d)	Drinking water; 3% ethoxylated castor oil vehicle	LOEL: 0.2%	TCE metabolite-protein adducts formed by a cytochrome P450-mediated pathway were detected by fluorescence imunohistochemistry in the epithelia of corpus epididymis and in efferent ducts.
DuTeaux et al., 2004b	Rat, Sprague-Dawley, male, 3/group, or Simonson albino (UC-Davis), male, 3/group	0, 0.2, or 0.4% (0, 143, or 270 mg/kg/d) 14 d	Drinking water, 3% ethoxylated castor oil vehicle	LOAEL: 0.2%	Dose-dependent ↓ in ability of sperm to fertilize oocytes collected from untreated ♀s. Oxidative damage to sperm membrane in head and mid-piece was indicated by dose-related ↑ in oxidized proteins and lipid peroxidation.
Veeramachaneni et al., 2001	Rabbit, Dutch belted, females and offspring; 7−9 offspring/group	9.5- or 28.5-ppm TCE [d] GD 20 thru lactation, then to offspring thru postnatal wk 15	Drinking water	LOAEL: 9.5 ppm	Decreased copulatory behavior; acrosomal dysgenesis, nuclear malformations; sig. ↓ LH and testosterone.
Zenick et al., 1984	Rat, Long-Evans, male, 10/group	0, 10, 100, or 1,000 mg/kg/d 6 wk, 5 d/wk; 4 wks recovery	Gavage, corn oil vehicle	NOAEL: 100 mg/kg/d LOAEL: 1,000 mg/kg/d	At 1,000 mg/kg, BW ↓, liver/BW ratios↑, and impaired copulatory behavior. Copulatory performance returned to normal by 5th wk of exposure. At wk 6, TCE and metabolites concentrated to a significant extent in male reproductive organs.
Studies assessing female reproductive outcomes					
Berger and Horner, 2003	Rat, Simonson (S-D derived), female, (5−6) × 3/group	0 or 0.45% 2 wks	Drinking water, 3% Tween vehicle	LOAEL: 0.45%	*In vitro* fertilization and sperm penetration of oocytes sig. ↓ with sperm harvested from untreated males.
Cosby and Dukelow, 1992	Mouse, B6D2F1, female, 7−12/group	0, 24, or 240 mg/kg/d GD 1−5, 6−10, or 11−15	Gavage, corn oil vehicle	NOAEL: 240 mg/kg/d	No treatment-related effects on *in vitro* fertilization in dams or offspring.

1

Table 4-76. Summary of mammalian *in vivo* reproductive toxicity studies—oral exposures (continued)

Reference	Species/strain/ sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL [a]	Effects
Manson et al., 1984	Rat, Long-Evans, female, 23−25/group	0, 10, 100, or 1,000 mg/kg/d 6 wks: 2 wk premating, 1 wk mating period, GD 1−21	Gavage, corn oil vehicle	NOAEL: 100 mg/kg/d LOAEL: 1,000 mg/kg/d	Female fertility and mating success was not affected. At 1,000 mg/kg/d group, 5/23 females died, gestation BW gain was sig. ↓. After subchronic oral TCE exposure, TCE was detected in fat, adrenals, and ovaries; TCA levels in uterine tissue were high. At 1,000 mg/kg/d, neonatal deaths (female pups) were ↑ on PNDs 1, 10, and 14. Dose-related ↑ seen in TCA in blood, liver and milk in stomach of ♀ pups, not ♂s.
Wu and Berger, 2007	Rat, Simonson (S-D derived), female, (no./group not reported)	0 or 0.45% (0.66 g/kg-d) [b] Preovulation days 1−5, 6−10, 11−14, or 1−14	Drinking water, 3% Tween vehicle	LOAEL: 0.45%	*In vitro* fertilization and sperm penetration of oocytes sig. ↓ with sperm harvested from untreated males.
Wu and Berger, 2008	Rat, Simonson (S-D derived), female, (no./group not reported)	0 or 0.45% (0.66 g/kg-d) [b] 1 or 5 d	Drinking water, 3% Tween vehicle	NOEL: 0.45%	Ovarian mRNA expression for ALCAM and Cudz1 protein were not altered.

Table 4-76. Summary of mammalian *in vivo* reproductive toxicity studies—oral exposures (continued)

Reference	Species/strain/ sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL [a]	Effects
Studies assessing fertility and reproductive outcome in both sexes					
George et al., 1985	Mouse, CD-1, male and female, 20 pairs/treatment group; 40 controls/sex	0, 0.15, 0.30, or 0.60% [c] micro-encapsulated TCE (TWA dose estimates: 0, 173, 362, or 737 mg/kg/d) [b] Breeders exposed 1 wk premating, then for 13 wk; pregnant females throughout gestation (i.e., 18 wk total)	Dietary	Parental systemic toxicity: NOAEL: 0.30% LOAEL: 0.60%	At 0.60%, in F0: sig.↑ liver weights in both sexes; sig. ↓ testis and seminal vesicle weight; histopathology of liver and kidney in both sexes. At 0.60%, in F1: sig. ↓ BW on PND 74, and in postpartum F1 dams; sig. ↑ liver, testis, and epididymis weights in males, sig. ↑ kidney weights in both sexes; sig. ↓ testis and seminal vesicle weight; histopathology of liver and kidney in both sexes.
				Parental reproductive function: LOAEL: 0.60% c	At 0.60%, in F0 and F1 males: sig. ↓ sperm motility.
				Offspring toxicity: NOAEL: 0.30% LOAEL: 0.60%	At 0.60%, in F1 pups: sig. ↓ live birth weights, sig. ↓ PND 4 pup BW; perinatal mortality ↑ (PND 0−21).
George et al., 1986	Rat, F334, males and female, 20 pairs/ treatment group, 40 controls/sex	0, 0.15, 0.30 or 0.60% [c] micro-encapsulated TCE Breeders exposed 1 wk premating, then for 13 wk; pregnant females throughout gestation (i.e., 18 wk total)	Dietary	Parental systemic toxicity: LOAEL: 0.15%	At 0.60%, in F0: sig. ↓ postpartum dam BW; sig. ↓ term. BW in both sexes; sig. ↑ liver, and kidney/adrenal weights in both sexes; sig. ↑ testis/epididymis weights; in F1: sig. ↓ testis weight. At all doses in F1: sig. ↓ postpartum dam BW; sig.↓ term. BW in both sexes, sig. ↑ liver wt. in both sexes. At 0.30 and 0.60%, in F1: sig. ↑ liver wt. in females.

Table 4-76. Summary of mammalian *in vivo* reproductive toxicity studies—oral exposures (continued)

Reference	Species/strain/sex/number	Dose level/exposure duration	Route/vehicle	NOAEL; LOAEL [a]	Effects
George et al., 1986 (continued)				Parental reproductive function: LOAEL: 0.60% [c]	At 0.60%, sig ↓ mating in F0 males and females (in cross-over mating trials).
				Offspring toxicity: LOAEL: 0.15%	At 0.60%, sig. ↓ F1 BW on PND 4 and 14. At all doses, sig. ↓ F1 BW on PND 21 and 80. At 0.3 and 0.60%, sig. ↓ live F1 pups/litter. At 0.15 and 0.60%, trend toward ↓ F1 survival from PND 21 to PND 80.

[a]NOAEL, LOAEL, NOEL, and LOEL (lowest-observed-effect level) are based upon reported study findings.
[b]Dose conversion calculations by study author(s).
[c]Fertility and reproduction assessment of last litter from continuous breeding phase and cross-over mating assessment (rats only) were conducted for 0 or 0.60% dose groups only.
[d]Concurrent exposure to several ground water contaminants; values given are for TCE levels in the mixture.

4.8.1.2.1. ***Inhalation exposures.*** Studies in rodents exposed to TCE via inhalation are described below and summarized in Table 4-75. These studies focused on various aspects of male reproductive organ integrity, spermatogenesis, or sperm function in rats or mice. In the studies published after the year 2000, the effects of either 376 or 1,000-ppm TCE were studied following exposure durations ranging from 1 to 24 weeks, and adverse effects on male reproductive endpoints were observed.

Kumar et al. (2000a) exposed male Wistar rats in whole body inhalation chambers to 376-ppm TCE for 4 hours/day, 5 days/week over several duration scenarios. These were 2-weeks (to observe the effect on the epididymal sperm maturation phase), 10 weeks (to observe the effect on the entire spermatogenic cycle), 5 weeks with 2 weeks rest (to observe the effect on primary spermatocytes differentiation to sperm), 8 weeks with 5 weeks rest (to observe effects on an intermediate stage of spermatogenesis), and 10 weeks with 8 weeks rest (to observe the effect on spermatogonial differentiation to sperm). Control rats were exposed to ambient air. Weekly mating with untreated females was conducted. At the end of the treatment/rest periods, the animals were sacrificed; testes and cauda epididymes tissues were collected. Alterations in testes histopathology (smaller, necrotic spermatogenic tubules), increased sperm abnormalities,

and significantly increased pre- and/or postimplantation loss in litters were observed in the groups with 2 or 10 weeks of exposure, or 5 weeks of exposure with 2 weeks rest. It was hypothesized that postmeiotic cells of spermatogenesis and epididymal sperm were affected by TCE exposure, leading to reproductive impairment.

To test the hypothesis that TCE exposure adversely affects sperm function and fertilization, Xu et al. (2004) conducted a study in which male CD-1 mice were exposed by inhalation to atmospheres containing 1,000 ppm (5.37 mg/L) TCE for 1 to 6 weeks (6 hours/day, 5 days/week). After each TCE exposure, body weights were recorded. Following termination, the right testis and epididymis of each treated male were weighed, and sperm was collected from the left epididymis and vas deferens for assessment of the number of total sperm and motile sperm. Sperm function was evaluated in the following experiments: (1) suspensions of capacitated vas deferens/cauda epididymal sperm were examined for spontaneous acrosome reaction, (2) *in vitro* binding of capacitated sperm to mature eggs from female CF-1 mice (expressed as the number of sperm bound per egg) was assessed, and (3) *in vivo* fertilization was evaluated via mating of male mice to superovulated female CF-1 mice immediately following inhalation exposure; cumulus masses containing mature eggs were collected from the oviducts of the females, and the percentage of eggs fertilized was examined. Inhalation exposure to TCE did not result in altered body weight, testis and epididymis weights, sperm count, or sperm morphology or motility. Percentages of acrosome-intact sperm populations were similar between treated and control animals. Nevertheless, for males treated with TCE for 2 or more weeks decreases were observed in the number of sperm bound to the oocytes *in vitro* (significant at 2 and 6 weeks, $p < 0.001$). In a follow-up assessment, control sperm were incubated for 30-minutes in buffered solutions of TCE or metabolites (chloral hydrate or trichloroethanol); while TCE-incubation had no effect on sperm-oocyte binding, decreased binding capacity was noted for the metabolite-incubated sperm. The ability for sperm from TCE-exposed males to bind to and fertilize oocytes *in vivo* was also found to be significantly impaired ($p < 0.05$).

A study designed to investigate the role of testosterone, and of cholesterol and ascorbic acid (which are primary precursors of testosterone) in TCE-exposed rats with compromised reproductive function was conducted by Kumar et al. (2000b). Male Wistar rats (12−13/group) were exposed (whole body) to 376 ppm TCE by inhalation for 4 hours/day, 5 days/week, for either 12 or 24 weeks and then terminated. Separate ambient-air control groups were conducted for the 12- and 24-week exposure studies. Epididymal sperm count and motility were evaluated, and measures of 17-β-hydroxy steroid dehydrogenase (17-β-HSD), testicular total cholesterol and ascorbic acid, serum testosterone, and glucose 6-p dehydrogenase (G6PDH) in testicular homogenate were assayed. In rats exposed to TCE for either 12 or 24 weeks, total epididymal sperm count and motility, serum testosterone concentration, and specific activities of both

17-β-HSD and G6PDH were significantly decreased ($p < 0.05$), while total cholesterol content was significantly ($p < 0.05$) increased. Ascorbic acid levels were not affected.

In another study, Kumar et al. (2001) utilized the same exposure paradigm to examine cauda epididymal sperm count and motility, testicular histopathology, and testicular marker enzymes: sorbitol dehydrogenase (SDH), G6PDH, glutamyl transferase (GT), and glucuronidase, in Wistar rats (6/group). After 24 weeks of exposure, testes weights and epididymal sperm count and motility were significantly decreased ($p < 0.05$). After 12 weeks of TCE exposure, histopathological examination of the testes revealed a reduced number of spermatogenic cells in the seminiferous tubules, fewer spermatids as compared to controls, and the presence of necrotic spermatogenic cells. Testicular atrophy, smaller tubules, hyperplastic Leydig cells, and a lack of spermatocytes and spermatids in the tubules were observed after 24 weeks of TCE exposure. After both 12 and 24 weeks of exposure, SDH and G6PDH were significantly ($p < 0.05$) reduced while GT and β-glucuronidase were significantly ($p < 0.05$) increased.

In a study by Land et al. (1981), 8−10 week old male mice (C57BlxC3H)F1 (5 or 10/group) were exposed (whole body) by inhalation to a number of anesthetic agents for 5 consecutive days at 4 hours/day and sacrificed 28 days after the first day of exposure. Chamber concentration levels for the TCE groups were 0.02 and 0.2%. The control group received ambient air. Epididymal sperm were evaluated for morphological abnormalities. At 0.2% TCE, the percent abnormal sperm in a sample of 1,000 was significantly ($p < 0.01$) increased as compared to control mice; no treatment-related effect on sperm morphology was observed at 0.02% TCE.

Forkert et al. (2002) exposed male CD-1 mice by inhalation to 1,000-ppm TCE (6 hours/day, 5 day/week) for 4 consecutive weeks and observed sloughing of portions of the epithelium upon histopathological evaluation of testicular and epididymal tissues.

Kan et al. (2007) also demonstrated that damage to the epididymal epithelium and sperm of CD-1 mice (4/group) resulted from exposure to 0 or 1,000-ppm TCE by inhalation for 6 hours/day, 5 days/week, for 1 to 4 weeks. Segments of the epididymis (caput, corpus, and cauda) were examined by light and electron microscope. As early as 1 week after TCE exposure, degeneration and sloughing of epithelial cells from all three epididymal areas were observed by light microscopy; these findings became more pronounced by 4 weeks of exposure. Vesiculation in the cytoplasm, disintegration of basolateral cell membranes, and epithelial cell sloughing were observed with electron microscopy. Sperm were found *in situ* in the cytoplasm of degenerated epididymal cells. A large number of sperm in the lumen of the epididymis were abnormal, including head and tail abnormalities.

4.8.1.2.2. ***Oral exposures.*** A variety of studies were conducted to assess various aspects of male and/or female reproductive capacity in laboratory animal species following oral exposures to TCE. These are described below and summarized in Table 4-76. They include studies that focused on male reproductive outcomes in rats or rabbits following gavage or drinking water exposures (Zenick et al., 1984; DuTeaux et al., 2003, 2004b; Veeramachaneni et al., 2001), studies that focused on female reproductive outcomes in rats following gavage or drinking water exposures (Berger and Horner, 2003; Cosby and Dukelow, 1992; Manson et al., 1984; Wu and Berger, 2007, 2008), and studies assessed fertility and reproductive outcome in both sexes following dietary exposures to CD-1 mice or F344 rats (George et al., 1985, 1986).

4.8.1.2.2.1. ***Studies assessing male reproductive outcomes.*** Zenick et al. (1984) conducted a study in which sexually experienced Long-Evans hooded male rats were administered 0, 10, 100, or 1,000 mg/kg/d TCE by gavage in corn oil for 6 weeks. A 4-week recovery phase was also incorporated into the study design. Endpoints assessed on Weeks 1 and 5 of treatment included copulatory behavior, ejaculatory plug weights, and ejaculated or epididymal sperm measures (count, motility, and morphology). Sperm measures and plug weights were not affected by treatment, nor were Week 6 plasma testosterone levels found to be altered. TCE effects on copulatory behavior (ejaculation latency, number of mounts, and number of intromissions) were observed at 1,000 mg/kg/d; these effects were recovered by 1−4 weeks post-treatment. Although the effects on male sexual behavior in this study were believed to be unrelated to narcotic effects of TCE, a later study by Nelson and Zenick (1986) showed that naltrexone (an opioid receptor antagonist, 2.0 mg/kg, i.p., administered 15 minutes prior to testing) could block the effect. Thus, it was hypothesized that the adverse effects of TCE on male copulatory behavior in the rat at 1,000 ppm may in fact be mediated by the endogenous opioid system at the CNS level.

In a series of experiments by DuTeaux et al. (2003, 2004b), adult male rats were administered 0, 0.2, or 0.4% TCE (v/v) (equivalent to 0, 2.73 mg/L, or 5.46 mg/L) in a solution of 3% ethoxylated castor oil in drinking water for 14 days. These concentrations were within the range of measurements obtained in formerly contaminated drinking water wells, as reported by ATSDR (1997). The average ingested doses of TCE (based upon animal body weight and average daily water consumption of 28 mL) were calculated to be 143 or 270 mg/kg/d for the low and high-dose groups, respectively (DuTeaux et al., 2008). Cauda epididymal and vas deferens sperm from treated males were incubated in culture medium with oviductal cumulus masses from untreated females to assess *in vitro* fertilization capability. Treatment with TCE resulted in a dose-dependent decrease in the ability of sperm to fertilize oocytes. Terminal body weights and testis/epididymal weights were similar between control and treated groups. Evaluation of sperm concentration or motility parameters did not reveal any treatment-related

alterations; acrosomal stability and mitochondrial membrane potential were not affected by treatment. Although no histopathological changes were observed in the testis or in the caput, corpus, or cauda epididymis, exposure to 0.2 and 0.4% TCE resulted in slight cellular alterations in the efferent ductule epithelium.

Veeramachaneni et al. (2001) evaluated the effects of drinking water containing chemicals typical of ground water near hazardous waste sites (including 9.5- or 28.5-ppm TCE) on male reproduction. In this study, pregnant Dutch-belted rabbits were administered treated drinking water from gestation Day 20; treatment continued through the lactation period and to weaned offspring (7−9/group) through postnatal Week 15. Deionized water was administered from postnatal weeks 16−61, at which time the animals were terminated. At 57−61 weeks of age, ejaculatory capability, and seminal, testicular, epididymal, and endocrine characteristics were evaluated. In both treated groups, long-term effects consisted of decreased copulatory behavior (interest, erection, and/or ejaculation), significant increases in acrosomal dysgenesis and nuclear malformations ($p < 0.03$), and significant decreases in serum concentration of luteinizing hormone ($p < 0.05$) and testosterone secretion after human chorionic gonadotropin administration ($p < 0.04$). There were no effects on total spermatozoa per ejaculate or on daily sperm production. The contribution of individual drinking water contaminants to adverse male reproductive outcome could not be discerned in this study. Additionally, it was not designed to distinguish between adverse effects that may have resulted from exposures in late gestation (i.e., during critical period of male reproductive system development) versus postnatal life.

4.8.1.2.2.2. ***Studies assessing female reproductive outcomes*****.** In a study that evaluated postnatal growth following gestational exposures, female B6D2F1 mice (7−12/group) were administered TCE at doses of 0, 1% LD_{50} (24 mg/kg/d), and 10% LD_{50} (240 mg/kg/d) by gavage in corn oil from gestation days 1−5, 6−10, or 11−15 (day of mating was defined as gestation Day 1) (Cosby and Dukelow, 1992). Litters were examined for pup count, sex, weight, and crown-rump measurement until postnatal Day 21. Some offspring were retained to 6 weeks of age, at which time they were killed and the gonads were removed, weighed and preserved. No treatment-related effects were observed in the dams or offspring. In a second series of studies conducted by Cosby and Dukelow and reported in the same paper, TCE and its metabolites DCA, TCA, and TCOH were added to culture media with capacitated sperm and cumulus masses from B6D2F1 mice to assess effects on *in vitro* fertilization. Dose-related decreases in fertilization were observed for DCA, TCA, and TCOH at 100 and 1,000 ppm, but not with TCE. Synergystic effects were not observed with TCA and TCOH.

A study was conducted by Manson et al. (1984) to determine if subchronic oral exposure to TCE affected female reproductive performance, and if TCE or its metabolites trichloroacetic

acid or trichloroethanol accumulated in female reproductive organs or neonatal tissues. Female Long-Evans hooded rats (22−23/group) were administered 0 (corn oil vehicle), 10, 100, or 1,000 mg/kg/d of TCE by gavage for 2 weeks prior to mating, throughout mating, and to gestation Day 21. Delivered pups were examined for gross anomalies, and body weight and survival were monitored for 31 days. Three maternal animals per group and 8−10 neonates per group (killed on postnatal Days 3 and 31) were analyzed for TCE and metabolite levels in tissues. TCE exposure resulted in 5 deaths and decreased maternal body weight gain at 1,000 mg/kg/d, but did not affect estrous cycle length or female fertility at any dose level. There were no evident developmental anomalies observed at any treatment level; however, at 1,000 mg/kg/d there was a significant increase in the number of pups (mostly female) born dead, and the cumulative neonatal survival count through PND 18 was significantly decreased as compared to control. TCE levels were uniformly high in fat, adrenal glands, and ovaries across treatment groups, and TCA levels were high in uterine tissue. TCE levels in the blood, liver, and milk contents of the stomach increased in female PND-3 neonates across treatment groups. These findings suggest that increased metabolite levels did not influence fertility, mating success, or pregnancy outcome.

In another study that examined the potential effect of TCE on female reproductive function, Berger and Horner (2003) conducted 2-week exposures of Sprague-Dawley derived female Simonson rats to tetrachloroethylene, trichloroethylene, several ethers, and 4-vinylcyclohexene diepoxide in separate groups. The TCE-treated group received 0.45% TCE in drinking water containing 3% Tween vehicle; control groups were administered either untreated water, or water containing the 3% Tween vehicle. There were 5−6 females/group, and three replicates were conducted for each group. At the end of exposure, ovulation was induced, the rats were killed, and the ovaries were removed. The zona pellucida was removed from dissected oocytes, which were then placed into culture medium and inseminated with sperm from untreated males. TCE treatment did not affect female body weight gain, the percentage of females ovulating, or the number of oocytes per ovulating female. Fertilizability of the oocytes from treated females was reduced significantly (46% for TCE-treated females versus 56% for vehicle controls). Oocytes from TCE-treated females had reduced ability to bind sperm plasma membrane proteins compared with vehicle controls.

In subsequent studies, Wu and Berger (2007, 2008) examined the effect of TCE on oocyte fertilizibility and ovarian gene expression. TCE was administered to female Simonson rats (number of subjects not reported) in the drinking water at 0 or 0.45% (in 3% Tween vehicle); daily doses were estimated to be 0.66 g TCE/kg body weight/day. In the oocyte fertilizibility study (Wu and Berger, 2007), the female rats were treated on Days 1−5, 6−10, 11−14, or 1−14 of the 2-week period preceding ovulation (on Day 15). Oocytes were extracted and fertilized *in*

vitro with sperm from a single male donor rat. With any duration of TCE exposure, fertilization (as assessed by the presence of decondensed sperm heads) was significantly ($p < 0.05$) decreased as compared to controls. After exposure on Days 6−10, 11−14, or 1−14, the oocytes from TCE-treated females had a significantly decreased ability to bind sperm ($p < 0.05$) in comparison to oocytes from vehicle controls. Increased protein carbonyls (an indicator of oxidatively modified proteins) were detected in the granulosa cells of ovaries from females exposed to TCE for 2 weeks. The presence of oxidized protein was confirmed by Western blot analysis. Microsomal preparations demonstrated the localization of cytochrome P450 2E1 and glutathione s-transferase (TCE-metabolizing enzymes) in the ovary. Ovarian mRNA transcription for ALCAM and Cuzd1 protein was not found to be altered after 1 or 5 days of exposure (Wu and Berger, 2008), suggesting that the post-translational modification of proteins within the ovary may partially explain the observed reductions in oocyte fertilization.

4.8.1.2.2.3. ***Studies assessing fertility and reproductive outcomes in both sexes*.** Assessments of reproduction and fertility with continuous breeding were conducted in NTP studies in CD-1 mice (George et al., 1985) and Fischer 344 rats (George et al., 1986). TCE was administered to the mice and rats at dietary levels of 0, 0.15, 0.30, or 0.60%, based upon the results of preliminary 14-day dose-range finding toxicity studies. Actual daily intake levels for the study in mice were calculated from the results of dietary formulation analyses and body weight/food consumption data at several time points during study conduct; the most conservative were from the second week of the continuous breeding study: 0, 52.5, 266.3, and 615.0 mg/kg/d. No intake calculations were presented for the rat study. In these studies, which were designed as described by Chapin and Sloane (1996), the continuous breeding phase in F0 adults consisted of a 7-day premating exposure, 98-day cohabitation period, and 28-day segregation period. In rats, a crossover mating trial (i.e., control males × control females; 0.60% TCE males × control females; control males × 0.60% TCE females) was conducted to further elucidate treatment-related adverse reproductive trends observed in the continuous breeding phase. The last litter of the continuous breeding phase was raised to sexual maturity for an assessment of fertility and reproduction in control and high-dose groups; for the rats, this included an open field behavioral assessment of F1 pups. The study protocol included terminal studies in both generations, including sperm evaluation (count morphology, and motility) in 10 selected males per dose level, macroscopic pathology, organ weights, and histopathology of selected organs.

In the continuous breeding phase of the CD-1 mouse study (George et al., 1985), no clinical signs of toxicity were observed in the parental (F0) animals, and there were no treatment-related effects on the proportion of breeding pairs able to produce a litter, the number of live pups per litter, the percent born live, the proportion of pups born live, the sex of pups born live,

absolute live pup weights, or adjusted female pup weights. At the high dose level of 0.60%, a number of adverse outcomes were observed. In the parental animals, absolute and body-weight-adjusted male and female liver weight values were significantly increased ($p < 0.01$), and right testis and seminal vesicle weights were decreased ($p < 0.05$), but kidney/adrenal weights were not affected. Sperm motility was significantly ($p < 0.01$) decreased by 45% in treated males as compared to controls. Histopathology examination revealed lesions in the liver (hypertrophy of the centrilobular liver cells) and kidneys (tubular degeneration and karyomegaly of the corticomedullary renal tubular epithelium) of F0 males and females. In the pups at 0.60%, adjusted live birth weights for males and both sexes combined were significantly decreased ($p < 0.01$) as compared to control. The last control and high-dose litters of the continuous breeding assessment were raised to the age of sexual maturity for a further assessment of reproductive performance. In these F1 pups, body weights (both sexes) were significantly decreased at PND 4, and male offspring body weights were significantly ($p < 0.05$) less than controls at PND 74 (±10). It was reported that perinatal mortality (PND 0−21) was increased, with a 61.3% mortality rate for TCE-treated pups versus a 28.3% mortality rate for control pups. Reproductive performance was not affected by treatment, and postmortem evaluations of the F1 adult mice revealed significant findings at 0.60% TCE that were consistent with those seen in the F0 adults and additionally demonstrated renal toxicity, i.e., elevated liver and kidney/adrenal weights and hepatic and renal histopathological lesions in both sexes, elevated testis and epididymis weights in males, and decreased sperm motility (18% less than control).

The F344 rat study continuous breeding phase demonstrated no evidence of treatment-related effects on the proportion of breeding pairs able to produce a litter, percent of pups born alive, the sex of pups born alive, or absolute or adjusted pup weights (George et al., 1986). However, the number of live pups per litter was significantly ($p < 0.05$) decreased at 0.30 and 0.60% TCE, and a significant ($p < 0.01$) trend toward a dose-related decrease in the number of live litters per pair was observed; individual data were reported to indicate a progressive decrease in the number of breeding pairs in each treatment group producing third, fourth, and fifth litters. The crossover mating trial conducted in order to pursue this outcome demonstrated that the proportion of detected matings was significantly depressed ($p < 0.05$) in the mating pairs with TCE-treated partners compared to the control pairs. In the F0 adults at 0.60% TCE, postpartum dam body weights were significantly decreased ($p < 0.01$ or 0.05) in the continuous breeding phase and the crossover mating trials, and terminal body weights were significantly decreased ($p < 0.01$) for both male and female rats. Postmortem findings for F0 adults in the high-dose group included significantly increased absolute and body-weight-adjusted liver and kidney/adrenal weights in males, increased adjusted liver and kidney/adrenal weights in females, and significantly increased adjusted left testis/epididymal weights. Sperm assessment did not

identify any effects on motility, concentration or morphology, and histopathological examination was negative. The last control and high-dose litters of the continuous breeding assessment were raised to the age of sexual maturity for assessment of open field behavior and reproductive performance. In these F1 pups at 0.60% TCE, body weights of male and females were significantly ($p < 0.05$ or 0.01, respectively) decreased at PND 4 and 14. By PND 21, pup weights in both sexes were significantly reduced in all treated groups, and this continued until termination (approximately PND 80). A tendency toward decreased postweaning survival (i.e., from PND 21 to PND 81 ± 10) was reported for F1 pups at the 0.15 and 0.60% levels. Open field testing revealed a significant ($p < 0.05$) dose-related trend toward an increase in the time required for male and female F1 weanling pups to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment. Reproductive performance assessments conducted in this study phase were not affected by treatment. Postpartum F1 dam body weights were significantly decreased ($p < 0.05$ or 0.01) in all of the TCE-treated groups as compared to controls, as were terminal body weights for both adult F1 males and females. Postmortem evaluations of the F1 adult rats revealed significantly ($p < 0.01$) decreased left testis/epididymis weight at 0.60% TCE, and significantly ($p < 0.05$ or 0.01) increased adjusted mean liver weight in all treated groups for males and at 0.30 and 0.60% for females. Sperm assessments for F1 males revealed a significant increase ($p < 0.05$) in the percent abnormal sperm in the 0.30% TCE group, but no other adverse effects on sperm motility, concentration, or morphology were observed. As with the F0 adults, there were no adverse treatment-related findings revealed at histopathological assessment. The study authors concluded that the observed effects to TCE exposure in this study were primarily due to generalized toxicity and not to a specific effect on the reproductive system; however, based upon the overall toxicological profile for TCE, which demonstrates that the male reproductive system is a target for TCE exposures, this conclusion is not supported.

4.8.1.3. *Discussion/Synthesis of noncancer reproductive toxicity findings*

The human epidemiological findings and animal study evidence consistently indicate that TCE exposures can result in adverse reproductive outcomes. Although the epidemiological data may not always be robust or unequivocal, they demonstrate the potential for a wide range of exposure-related adverse outcomes on female and male reproduction. In animal studies, there is some evidence for female-specific reproductive toxicity; but there is strong and compelling evidence for adverse effects of TCE exposure on male reproductive system and function.

4.8.1.3.1. ***Female reproductive toxicity.*** Although few epidemiological studies have examined TCE exposure in relation to female reproductive function (see Table 4-77), the available studies

provide evidence of decreased fertility, as measured by time to pregnancy (Sallmén et al., 1995), and effects on menstrual cycle patterns, including abnormal cycle length (ATSDR, 2001), amenorrhea (Sagawa et al., 1973; Zielinski, 1973), and menstrual "disturbance" (Bardodej and Vyskocil, 1956). In experimental animals, the effects on female reproduction include evidence of reduced *in vitro* oocyte fertilizability in rats (Berger and Horner, 2003; Wu and Berger, 2007). However, in other studies that assessed reproductive outcome in female rodents (Cosby and Dukelow, 1992; George et al., 1985, 1986; Manson et al., 1984), there was no evidence of adverse effects of TCE exposure on female reproductive function. Overall, although the data are suggestive, there are inadequate data to make conclusions as to whether adverse effects on human female reproduction are caused by TCE.

Table 4-77. Summary of adverse female reproductive outcomes associated with TCE exposures

Finding	Species	Citation
Menstrual cycle disturbance	Human	ATSDR, 2001[a]
		Bardodej and Vyskocil, 1956
		Sagawa et al., 1973
		Zielinski, 1973
Reduced fertility	Human[a]	Sallmén et al., 1995
	Rat[b]	Berger and Horner, 2003
		Wu and Berger, 2007

[a]Not significant.
[b]*In vitro* oocyte fertilizability.

4.8.1.3.2. ***Male reproductive toxicity.*** Notably, the results of a number of studies in both humans and experimental animals have suggested that exposure to TCE can result in targeted male reproductive toxicity (see Table 4-78). The adverse effects that have been observed in both male humans and male animal models include altered sperm count, morphology, or motility (Chia et al., 1996; George et al., 1985; Kumar et al, 2000a, b, 2001; Land et al., 1981; Rasmussen et al., 1988; Veeramachaneni et al., 2001); decreased libido or copulatory behavior (Bardodej and Vyskocil, 1956; El Ghawabi et al., 1973; George et al., 1986; Saihan et al., 1978; Veeramachaneni et al., 2001; Zenick et al., 1984); alterations in serum hormone levels (Chia et al., 1997; Goh et al., 1998; Kumar et al., 2000b; Veeramachaneni et al., 2001); and reduced fertility (George et al., 1986). However, other studies in humans did not see evidence of altered sperm count or morphology (Rasmussen et al., 1988) or reduced fertility (Forkert et al.,

2003; Sallmén et al., 1998), and some animal studies also did not identify altered sperm measures (Cosby and Dukelow, 1992; Xu et al., 2004; Zenick et al., 1984; George et al, 1986). Additional adverse effects observed in animals include histopathological lesions of the testes (George et al., 1986; Kumar et al., 2000a, 2001) or epidiymides (Forkert et al., 2002; Kan et al., 2007) and altered *in vitro* sperm-oocyte binding and/or *in vivo* fertilization for TCE and/or its metabolites (Xu et al., 2004; DuTeaux et al., 2004b).

In spite of the preponderance of studies demonstrating effects on sperm parameters, there is an absence of overwhelming evidence in the database of adverse effects of TCE on overall fertility in the rodent studies. That is not surprising, however, given the redundancy and efficiency of rodent reproductive capabilities. Nevertheless, the continuous breeding reproductive toxicity study in rats (George et al., 1986) did demonstrate a trend towards reproductive compromise (i.e., a progressive decrease in the number of breeding pairs producing third, fourth, and fifth litters).

It is noted that in the studies by George et al. (1985, 1986), adverse reproductive outcomes in male rats and mice were observed at the highest dose level tested (0.060% TCE in diet) which was also systemically toxic (i.e., demonstrating kidney toxicity and liver enzyme induction and toxicity, sometimes in conjunction with body weight deficits). Because of this, the study authors concluded that the observed reproductive toxicity was a secondary effect of generalized systemic toxicity; however, this conclusion is not supported by the overall toxicological profile of TCE which provides significant evidence indicating that TCE is a reproductive toxicant.

4.8.1.3.2.1. ***The role of metabolism in male reproductive toxicity.*** There has been particular focus on evidence of exposure to male reproductive organs by TCE and/or its metabolites, as well as the role of TCE metabolites in the observed toxic effects.

In humans, a few studies demonstrating male reproductive toxicity have measured levels of TCE in the body. U-TCA was measured in men employed in an electronics factory, and adverse effects observed included abnormal sperm morphology and hyperzoospermia and altered serum hormone levels (Chia et al., 1996, 1997; Goh et al., 1998). U-TCA was also measured as a marker of exposure to TCE in men occupationally exposed to solvents, although this study did not report any adverse effects on fertility (Sallmén et al., 1998).

Table 4-78. Summary of adverse male reproductive outcomes associated with TCE exposures

Finding	Species	Citation
Testicular toxicity/pathology	Rat	George et al., 1986
		Kumar et al., 2000a
		Kumar et al., 2001
	Mouse	Kan et al., 2007
Epididymal toxicity/pathology	Mouse	Forkert et al., 2002
Decreased sperm quantity/quality	Human	Chia et al., 1996
		Rasmussen et al., 1988[a]
	Rat	Kumar et al., 2000a, b, 2001
	Mouse	George et al., 1985
		Land et al., 1981
	Rabbit	Veeramachaneni et al., 2001
Altered *in vitro* sperm-oocyte binding or *in vivo* fertilization	Rat	DuTeaux et al., 2004b
	Mouse	Cosby and Dukelow, 1992[b]
		Xu et al., 2004[b]
Altered sexual drive or function	Human	El Ghawabi et al., 1973
		Saihan et al., 1978[c]
		Bardodej and Vyskocil, 1956
	Rat	George et al., 1986
		Zenick et al., 1984
	Rabbit	Veeramachaneni et al., 2001
Altered serum testosterone levels	Human	Chia et al., 1997[d]
		Goh et al., 1998[e]
	Rat	Kumar et al., 2000b
	Rabbit	Veeramachaneni et al., 2001
Reduced fertility	Rat	George et al., 1986
Gynaecomastia	Human	Saihan et al., 1978[c]

[a] Nonsignificant increase in percentage of two YFF in spermatozoa; no effect on sperm count or morphology.
[b] Observed with metabolite(s) of TCE only.
[c] Case study of one individual.
[d] Also observed altered levels of DHEAS, FSH, and SHBG.
[e] Also observed altered levels of SHBG.

In the study in Long-Evans male rats by Zenick et al. (1984), blood and tissue levels of TCE, TCA, and TCOH were measured in three rats/group following 6 weeks of gavage treatment at 0, 10, 100, and 1,000 mg/kg/d. Additionally the levels of TCE and metabolites were measured in seminal plugs recovered following copulation at Week 5. Marked increases in TCE levels were observed only at 1,000 mg/kg/d, in blood, muscle, adrenals, and seminal plugs. It was reported that dose-related increases in TCA and TCOH concentrations were observed in the organs evaluated, notably including the reproductive organs (epididymis, vas deferens, testis, prostate, and seminal vesicle), thus, creating a potential for interference with reproductive function.

This potential was explored further in a study by Forkert et al. (2002), in which male CD-1 mice were exposed by inhalation to 1,000-ppm TCE (6 hours/day, 5 day/week) for 4 consecutive weeks. Urine was obtained on Days 4, 9, 14, and 19 of exposure and analyzed for concentrations of TCE and TCOH. Microsomal preparations from the liver, testis and epididymis were used for immunoblotting, determining *p*-nitrophenol hydroxylase and CYP2E1 activities, and evaluating the microsomal metabolism of TCE.

Subsequent studies conducted by the same laboratory (Forkert et al., 2003) evaluated the potential of the male reproductive tract to accumulate TCE and its metabolites including chloral, TCOH, TCA, and DCA. Human seminal fluid and urine samples from eight mechanics diagnosed with clinical infertility and exposed to TCE occupationally were analyzed. Urine samples from two of the eight subjects contained TCA and/or TCOH, suggesting that TCE exposure and/or metabolism was low during the time just prior to sample collection. TCE, chloral, and TCOH were detected in seminal fluid samples from all eight subjects, while TCA was found in one subject, and DCA was found in two subjects. Additionally, TCE and its metabolites were assessed in the epididymis and testis of CD-1 mice (4/group) exposed by inhalation (6 hours/day, 5 days/week) to 1,000 ppm TCE for 1, 2, and 4 weeks. TCE, chloral, and TCOH were found in the epididymis at all timepoints, although TCOH levels were increased significantly (tripled) at four weeks of exposure. This study showed that the metabolic disposition of TCE in humans is similar to that in mice, indicating that the murine model is appropriate for investigating the effects of TCE-induced toxicity in the male reproductive system. These studies provide support for the premise that TCE is metabolized in the human reproductive tract, mainly in the epididymis, resulting in the production of metabolites that cause damage to the epididymal epithelium and affect the normal development of sperm.

Immunohistochemical experiments (Forkert et al., 2002) confirmed the presence of CYP2E1 in the epididymis and testis of mice; it was found to be localized in the testicular Leydig cells and the epididymal epithelium. Similar results were obtained with the immunohistochemical evaluation of human and primate tissue samples. CYP2E1 has been

previously shown by Lipscomb et al. (1998) to be the predominant CYP enzyme catalyzing the hepatic metabolism of TCE in both animals and rodents. These findings support the role of CYP2E1 in TCE metabolism in the male reproductive tract of humans, primates, and mice.

4.8.1.3.2.2. ***Mode of action for male reproductive toxicity*****.** A number of studies have been conducted to attempt to characterize various aspects of the mode of action for observed male reproductive outcomes.

Studies by Kumar et al. (2000b, 2001) suggest that perturbation of testosterone biosynthesis may have some role in testicular toxicity and altered sperm measures. Significant decreases in the activity of G6PDH and accumulation of cholesterol are suggestive of an alteration in testicular steroid biosynthesis. Increased testicular lipids, including cholesterol, have been noted for other testicular toxicants such as lead (Saxena et al., 1987), triethylenemelamine (Johnson et al., 1967), and quinalphos (Ray et al., 1987), in association with testicular degeneration and impaired spermatogenesis. Since testosterone has been shown to be essential for the progression of spermatogenesis (O'Donnell et al., 1994), alterations in testosterone production could be a key event in male reproductive dysfunction following TCE exposure. Additionally, the observed TCE-related reduction of 17-β-HSD, which is involved in the conversion of androstenedione to testosterone, has also been associated with male reproductive insufficiency following exposure to phthalate esters (Srivastava and Srivastava, 1991), quinalphos (Ray et al., 1987), and lead (Saxena et al., 1987). Reductions in SDH, which are primarily associated with the pachytene spermatocyte maturation of germinal epithelium, have been shown to be associated with depletion of germ cells (Mills and Means, 1970; Chapin et al., 1982), and the activity of G6PDH is greatest in premeiotic germ cells and Leydig cells of the interstitium (Blackshaw et al., 1970). The increased GT and glucuronidase observed following TCE exposures appear to be indicative of impaired Sertoli cell function (Hodgen and Sherins, 1973; Sherins and Hodgen, 1976). Based upon the conclusions of these studies, Kumar et al. (2001) hypothesized that the reduced activity of G6PDH and SDH in testes of TCE-exposed male rats is indicative of the depletion of germ cells, spermatogenic arrest, and impaired function of the Sertoli cells and Leydig cells of the interstitium.

In the series of experiments by DuTeaux et al. (2003, 2004b), protein dichloroacetyl adducts were found in the corpus epididymis and in the efferent ducts of rats administered TCE; this effect was also demonstrated following *in vitro* exposure of reproductive tissues to TCE. Oxidized proteins were detected on the surface of spermatozoa from TCE-treated rats in a dose-response pattern; this was confirmed using a Western blotting technique. Soluble (but not mitochondrial) cysteine-conjugate β-lyase was detected in the epididymis and efferent ducts of treated rats. Following a single intraperitoneal injection of DCVC, no dichloroacetylated protein

adducts were detected in the epididymis and efferent ducts. The presence of CYP2E1 was found in epididymis and efferent ducts, suggesting a role of cytochrome P450–dependent metabolism in adduct formation. An *in vitro* assay was used to demonstrate that epididymal and efferent duct microsomes are capable of metabolizing TCE; TCE metabolism in the efferent ducts was found to be inhibited by anti-CYP2E1 antibody. Lipid peroxidation in sperm, presumably initiated by free radicals, was increased in a significant ($p < 0.005$) dose-dependent manner after TCE-exposure.

Overall, it has been suggested (DuTeaux et al., 2004b) that reproductive organ toxicities observed following TCE exposure are initiated by metabolic bioactivation, leading to subsequent protein adduct formation. It has been hypothesized that epoxide hydrolases in the rat epididymis may play a role in the biological activation of metabolites (DuTeaux et al., 2004a).

4.8.1.3.3. ***Summary of noncancer reproductive toxicity.*** The toxicological database for TCE includes a number of studies that demonstrate adverse effects on the integrity and function of the reproductive system in females and males. Both the epidemiological and animal toxicology databases provide suggestive, but limited, evidence of adverse outcomes to female reproductive outcomes. However, much more extensive evidence exists in support of an association between TCE exposures and male reproductive toxicity. The available epidemiological data and case reports that associate TCE with adverse effects on male reproductive function are limited in size and provide little quantitative dose data (Lamb and Hentz, 2006). However, the animal data provide extensive evidence of TCE-related male reproductive toxicity. Strengths of the database include the presence of both functional and structural outcomes, similarities in adverse treatment-related effects observed in multiple species, and evidence that metabolism of TCE in male reproductive tract tissues is associated with adverse effects on sperm measures in both humans and animals (suggesting that the murine model is appropriate for extrapolation to human health risk assessment). Additionally some aspects of a putative MOA (e.g., perturbations in testosterone biosynthesis) appear to have some commonalities between humans and animals.

4.8.2. Cancers of the Reproductive System

The effects of TCE on cancers of the reproductive system have been examined for males and females in both epidemiological and experimental animal studies. The epidemiological literature includes data on prostate in males and cancers of the breast and cervix in females. The experimental animal literature includes data on prostate and testes in male rodents; and uterus, ovary, mammary gland, vulva, and genital tract in female rodents. The evidence for these cancers is generally not robust.

4.8.2.1. *Human Data*

The epidemiologic evidence on TCE and cancer of the prostate, breast, and cervix is from cohort and geographic based studies. Two additional case-control studies of prostate cancer in males are nested within cohorts (Greenland et al., 1994; Krishnadasan et al., 2007). The nested case-control studies are identified in Tables 4-79−4-81 with cohort studies given their source population for case and control identification. One population-based case-control study examined on TCE exposure and prostate (Siemiatycki, 1991); however, no population case-control studies on breast or cervical cancers and TCE exposure were found in the peer-reviewed literature.

4.8.2.1.1. ***Prostate cancer.*** Sixteen cohort or PMR studies, two nested case-control, one population case-control, and two geographic-based studies present relative risk estimates for prostate cancer (Wilcosky et al., 1984; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Axelson et al., 1994; Siemiatycki, 1991; Greenland et al., 1994; Anttila et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999, 2006; Ritz, 1999; Hansen et al., 2001; Morgan and Cassady, 2002; Raaschou-Nielsen et al., 2003; Chang et al., 2003, 2005; ATSDR, 2004, 2006; Krishnadasan et al., 2007; Radican et al., 2008). Three small cohort studies (Costa et al., 1989; Sinks et al., 1992; Henschler et al., 1995), one multiple-site population case-control (Siemiatycki, 1991) and one geographic based study (Vartiainen et al., 1993) do not report estimates for prostate cancer in their published papers. Twelve of the 19 studies with prostate cancer relative risk estimates had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Siemiatycki, 1991; Axelson et al., 1994; Anttila et al., 1994; Greenland et al., 1994, Blair et al., 1998; Morgan et al., 1998, 2000; Boice et al., 1999, 2006; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Krishnadasan et al., 2007; Radican et al., 2008). Krishnadasan et al. (2007) in their nested case-control study of prostate cancer observed a 2-fold odds ratio estimate with high cumulative TCE exposure score (2.4, 95% CI: 1.3, 4.4, 20 year lagged exposure) and an increasing positive relationship between prostate cancer incidence and TCE cumulative exposure score ($p = 0.02$). TCE exposure was positively correlated with several other occupational exposures, and Krishnadasan et al. (2007) adjusted for possible confounding from all other chemical exposures as well as age at diagnosis, occupational physical activity, and socio-economic status in statistical analyses. Relative risk estimates in studies other than Krishnadasan et al. (2007) were above 1.0 for overall TCE exposure (1.8, 95% CI: 0.8, 4.0 [Siemiatycki, 1991]; 1.1, 95% CI: 0.6, 1.8 [Blair et al., 1998] and 1.20, 95% CI: 0.92, 1.76, with an additional 10-year follow-up [Radican et al., 2008]; 1.58, 95% CI: 0.96, 2.62 [Morgan et al., 1998, 2000; Environmental Health Strategies, 1997]; 1.3, 95% CI: 0.52, 2.69 [Boice et al.,

1999]; 1.38, 95% CI: 0.73, 2.35 [Anttila et al., 1995]) and prostate cancer risks did not appear to increase with increasing exposure. Four studies observed relative risk estimates below 1.0 for overall TCE exposure (0.93, 95% CI: 0.60, 1.37 [Garabrant et al., 1988]; 0.6, 95% CI: 0.2, 1.30 [Hansen et al., 2001]; 0.9, 95% CI: 0.79, 1.08 [Raaschou-Nielsen et al., 2003]; 0.82, 95% CI: 0.36, 1.62 [Boice et al., 2006]), and are not considered inconsistent because alternative explanations are possible and included observations are based on few subjects, lowering statistical power, or to poorer exposure assessment approaches that may result in a higher likelihood of exposure misclassification.

Seven other cohort, PMR, and geographic based studies were given less weight in the analysis because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity (Wilcosky et al., 1984; Shannon et al., 1988; Blair et al., 1989; Morgan and Cassady, 2002; ATSDR, 2004, 2006; Chang et al., 2005). Chang et al. (2005) observed a statistically significant deficit in prostate cancer risk, based on one case, and an insensitive exposure assessment (0.14, 95% CI: 0.00, 0.76). Relative risks in the other five studies ranged from 0.62 (CI not presented in paper) (Wilcosky et al., 1984) to 1.11 (95% CI: 0.98, 1.25) (Morgan and Cassady, 2002).

Risk factors for prostate cancer include age, family history of prostate cancer, and ethnicity as causal with inadequate evidence for a relationship with smoking or alcohol (Wigle et al., 2008). All studies except Krishnadasan et al. (2007) were not able to adjust for possible confounding from other chemical exposures in the work environment. None of the studies including Krishnadasan et al. (2007) accounted for other well-established nonoccupational risk factors for prostate cancer such as race, prostate cancer screening and family history. There is limited evidence that physical activity may provide a protective effect for prostate cancer (Wigle et al., 2008). Krishnadasan et al. (2008) examined the effect of physical activity in the Rocketdyne aerospace cohort (Zhao et al., 2005; Krishnadasan et al., 2007). Their finding of a protective effect with high physical activity (0.55, 95% CI: 0.32, 0.95, p trend = 0.04) after control for TCE exposure provides additional evidence (Krishnadasan et al., 2008) and suggests underlying risk may be obscured in studies lacking adjustment for physical activity.

Table 4-79. Summary of human studies on TCE exposure and prostate cancer

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Studies	Exposure group	Relative risk (95% CI)	No. obs. Events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Krishnadasan et al., 2007
	Low/moderate TCE score	1.3 (0.81, 2.1)[a,b]	90	
	High TCE score	2.1 (1.2, 3.9)[a,b]	45	
	p for trend	0.02		
	Low/moderate TCE score	1.3 (0.81, 2.1)[a,c]		
	High TCE score	2.4 (1.3, 4.4)[a,c]		
	p for trend	0.01		
All employees at electronics factory (Taiwan)		0.14 (0.00, 0.76)[d]	1	Chang et al., 2005
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure	0.9 (0.79, 1.08)	163	
Biologically-monitored Danish workers				Hansen et al., 2001
	Any TCE exposure, females	0.6 (0,2, 1.3)	6	
Aircraft maintenance workers (Hill Air Force Base, UT)				Blair et al., 1998
	TCE subcohort	Not reported	158	
	Cumulative exposure			
	0	1.0[e]		
	<5 ppm-yr	1.1 (0.7, 1.6)	64	
	5−25 ppm-yr	1.0 (0.6, 1.6)	38	
	>25 ppm-yr	1.2 (0.8, 1.8)	56	
	TCE subcohort	1.2 (0.92, 1.76)	116	Radican et al. 2008
	Cumulative exposure			
	0	1.0[e]		
	<5 ppm-yr	1.03 (0.65, 1.62)	41	
	5-25 ppm-yr	1.33 (0.82, 2.15)	42	
	>25 ppm-yr	1.31 (0.84, 2.06)	43	
Biologically-monitored Finnish workers		1.38 (0.73, 2.35)	13	Anttila et al., 1995
	Mean air-TCE (Ikeda extrapolation			
	<6 ppm	1.43 (0.62, 2.82)	8	
	6+ ppm	0.68 (0.08, 2.44)	2	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	Exposed workers	Not reported		
Biologically-monitored Swedish workers		1.25 (0.84, 1.84)	26	Axelson et al., 1994
Cardboard manufacturing workers, Atlanta area, GA		Not reported		Sinks et al., 1992

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Table 4-79. Summary of human studies on TCE exposure and prostate cancer (continued)

Studies	Exposure group	Relative risk (95% CI)	No. obs. Events	Reference
Cohort and PMR-mortality				
Aerospace workers (Rocketdyne)				Boice et al., 2006
	Any TCE (utility/eng flush)	0.82 (0.36, 1.62)	8	
View-Master employees		1.69 (0.68, 3.48)[f]	8	ATSDR, 2004
All employees at electronics factory (Taiwan)		Not reported	0	Chang et al., 2003
Fernald workers				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration	0.91 (0.38, 2.18)[e,g]	10	
	Moderate TCE exposure, >2 yrs duration	1.44 (0.19, 11.4)[e,g]	1	
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure to TCE	1.31 (0.52, 2.69)	7	
	Routine-intermittent	Not reported		
Aerospace workers (Hughes)				Morgan et al., 1998, 2000
	TCE subcohort	1.18 (0.73, 1.80)	21	
	Low intensity (<50 ppm)	1.03 (0.51, 1.84)	7	
	High intensity (>50 ppm)	0.47 (0.15, 1.11)	14	
	TCE subcohort (Cox Analysis)			
	Never exposed	1.00[e]		
	Ever exposed	1.58 (0.96, 2.62)[h]		
	Peak			
	No/Low	1.00[e]		
	Medium/high	1.39 (0.80, 2.41)[h]		
	Cumulative			
	Referent	1.00[e]		
	Low	1.72 (0.78, 3.80)[h]		
	High	1.53 (0.85, 2.75)[h]		
Aircraft maintenance workers (Hill Air Force Base, UT)				Blair et al., 1998
	TCE subcohort	1.1 (0.6, 1.8)	54	
	Cumulative exposure			
	0	1.0[e]		
	<5 ppm-yr	0.9 (0.5, 1.8)	19	
	5−25 ppm-yr	1.0 (0.5, 2.1)	13	
	>25 ppm-yr	1.3 (0.7, 2.4)	22	
Cardboard manufacturing workers in Arnsburg, Germany				
	TCE exposed workers	Not reported		Henschler et al., 1995
Deaths reported to GE pension fund (Pittsfield, MA)		0.82 (0.46, 1.46)[a]	58	Greenland et al., 1994
Cardboard manufacturing workers, Atlanta area, GA		Not reported	0	Sinks et al., 1992

Table 4-79. Summary of human studies on TCE exposure and prostate cancer (continued)

Studies	Exposure group	Relative risk (95% CI)	No. obs. Events	Reference
U. S. Coast Guard employee				Blair et al., 1989
	Marine inspectors	1.06 (0.51, 1.95)	10	
	Noninspectors	0.57 (0.15, 1.45)	7	
Aircraft manufacturing plant employees (Italy)				Costa et al., 1989
Aircraft manufacturing plant employees (San Diego, CA)		0.93 (0.60, 1.37)	25	Garabrant et al., 1988
Lamp manufacturing workers (GE)		1.56 (0.63, 3.22)	7	Shannon et al., 1988
Rubber workers				Wilcosky et al., 1984
	Any TCE exposure	0.62 (not reported)	3	
Case-control studies				
Population of Montreal, Canada				Siemiatycki, 1991
	Any TCE exposure	1.1 (0.6, 2.1)[i]	11	
	Substantial TCE exposure	1.8 (0.8, 4.0)[i]	7	
Geographic based studies				
Residents in two study areas in Endicott, NY		1.05 (0.75, 1.43)	40	ATSDR, 2006
Residents of 13 census tracts in Redlands, CA		1.11 (0.98, 1.25)[j]	483	Morgan and Cassady, 2002
Finnish residents				Vartiainen et al., 1993
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		

[a]Odds ratio from nested case-control study.
[b]Odds ratio, zero lag.
[c]Odds ratio, 20 year lag.
[d]Chang et al. (2005) presents SIRs for a category site of all cancers of male genital organs.
[e]Internal referents, workers without TCE exposure.
[f]Proportional mortality ratio.
[g]Analysis for >2 years exposure duration and a lagged TCE exposure period of 15 years.
[h]Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies (1997) Final Report to Hughes Corporation (Communication from Paul A. Cammer, President, Trichloroethylene Issues Group to Cheryl Siegel Scott, U.S. EPA, December 22, 1997).
[i]90% confidence interval.
[j]99% confidence interval.

Table 4-80. Summary of human studies on TCE exposure and breast cancer

Studies	Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al., 2005
	Any TCE exposure	Not reported		
	Low cumulative TCE score			
	Medium cumulative TCE score			
	High TCE score			
	p for trend			
All employees at electronics factory (Taiwan)				
	Females	1.09 (0.96, 1.22)[a]	286	Sung et al., 2007
	Females	1.19 (1.03, 1.36)	215	Chang et al., 2005
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure, males	0.5 (0.06, 1.90)	2	
	Any exposure, females	1.1 (0.89, 1.24)	145	
Biologically-monitored Danish workers				Hansen et al., 2001
	Any TCE exposure, males		0 (0.2 exp)	
	Any TCE exposure, females	0.9 (0.2, 2.3)	4	
Aircraft maintenance workers (Hill Air Force Base, UT)				Blair et al., 1998
	TCE subcohort	Not reported	34	
	Females, cumulative exposure			
	0	1.0[b]		
	<5 ppm-yr	0.3 (0.1, 1.4)	20	
	5−25 ppm-yr	0.4 (0.1, 2.9)	11	
	>25 ppm-yr	0.4 (0.4, 1.2)	3	
Biologically-monitored Finnish workers		Not reported		Anttila et al., 1995
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	Exposed workers	Not reported		
Biologically-monitored Swedish workers		Not reported		Axelson et al., 1994
Cardboard manufacturing workers, Atlanta area, GA		Not reported		Sinks et al., 1992
Cohort and PMR-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	Not reported		Boice et al., 2006
	Any exposure to TCE	Not reported		Zhao et al., 2005
	Low cumulative TCE score	Not reported		
	Medium cumulative TCE score	Not reported		
	High TCE score	Not reported		
	p for trend			
View-Master employees				ATSDR, 2004
	Males		0 (0.05 exp)	
	Females	1.02 (0.67, 1.49)[c]	27	

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Table 4-80. Summary of human studies on TCE exposure and breast cancer (continued)

Studies	Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Fernald workers				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration	Not reported		
	Moderate TCE exposure, >2 yrs duration	Not reported		
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure to TCE	1.31 (0.52, 2.69)[d]	7	
	Routine-intermittent[a]	Not reported		
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	0.75 (0.43, 1.22)[d]	16	
	Low intensity (<50 ppm)	1.03 (0.51, 1.84)[d]	11	
	High intensity (>50 ppm)	0.47 (0.15, 1.11)[d]	5	
	TCE subcohort (Cox Analysis)			
	Never exposed	1.00[d]	NR	
	Ever exposed	0.94 (0.51, 1.75) [d,e]	NR	
	Peak			
	No/Low	1.00[d]		
	Medium/high	1.14 (0.48, 2.70)[d,e]	NR	
	Cumulative			
	Referent	1.00[b]		
	Low	1.20 (0.60, 2.40)[d,e]	NR	
	High	0.65 (0.25, 1.69)[d,e]	NR	
Aircraft maintenance workers (Hill Air Force Base, UT)				Blair et al., 1998
	TCE subcohort (females)	2.0 (0.9, 4.6)	20	
	Females, cumulative exposure			
	0	1.0[b]		
	<5 ppm-yr	2.4 (1.1, 5.2)	10	
	5−25 ppm-yr	1.2 (0.3, 5.4)	21	
	>25 ppm-yr	1.4 (0.6, 3.2)	8	
	Low level intermittent exposure	3.1 (1.5, 6.2)	15	
	Low level continuous exposure	3.4 (1.4, 8.0)	8	
	Frequent peaks	1.4 (0.7, 3.2)	10	
	TCE subcohort (females)	1.23 (0.73, 2.06)	26	Radican et al., 2008

Table 4-80. Summary of human studies on TCE exposure and breast cancer (continued)

Studies	Exposure group	Relative risk (95% CI)	No. obs. events	Reference
	Females, cumulative exposure			
	0	1.0 [b]		
	<5 ppm-yr	1.57 (0.81, 3.04)	12	
	5-25 ppm-yr	1.01 (0.31, 3.30)	3	
	>25 ppm-yr	1.05 (0.53, 2.07)	11	
	Low level intermittent exposure	1.92 (1.08, 3.43)	18	
	Low level continuous exposure	1.71 (0.79, 3.71)	8	
	Frequent peaks	1.08 (0.57, 2.02)	14	
Cardboard manufacturing workers in Arnsburg, Germany				
	TCE exposed workers	Not examined		Henschler et al., 1995
Deaths reported to GE pension fund (Pittsfield, MA)		Not reported		Greenland et al., 1994
Cardboard manufacturing workers, Atlanta area, GA		Not reported	0	Sinks et al., 1992
U. S. Coast Guard employees				Blair et al., 1989
	Marine inspectors	Not reported		
	Noninspectors	Not reported		
Aircraft manufacturing plant employees (Italy)		Not reported[f]		Costa et al., 1989
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects, females	0.81 (0.52, 1.48)[d]	16	
Lamp manufacturing workers (GE)				Shannon et al., 1988
	Coil/wire drawing	2.04 (0.88, 4.02)	8	
	Other areas	0.97 (0.57, 1.66)	13	
Case-control Studies				
Population of Montreal, Canada				Siemiatycki, 1991
	Any TCE exposure	Not reported		
	Substantial TCE exposure	Not reported		
Geographic Based Studies				
Residents in two study areas in Endicott, NY		0.88 (0.65, 1.18)	46	ATSDR, 2006
Residents of 13 census tracts in Redlands, CA		1.09 (0.97, 1.21)	536	Morgan and Cassady, 2002
Finnish residents				Vartiainen et al., 1993
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		

[a]15 year lag.
[b]Internal referents, workers not exposed to TCE.
[c] Proportional mortality ratio.
[d]In Garabramt et al. (1998), Morgan et al. (1998) and Boice et al. (1999), breast cancer risk is for males and females combined (ICD-9, 174, 175).
[e]Risk ratio from Cox Proportional Hazard Analysis, stratified by age and sex, from Environmental Health Strategies (1997) Final Report to Hughes Corporation (Communication from Paul A. Cammer, President, Trichloroethylene Issues Group to Cheryl Siegel Scott, U.S. EPA, December 22, 1997).
[f]The cohort of Blair et al. (1989) and Costa et al. (1989) are composed of males only.

NR = not reported

Table 4-81. Summary of human studies on TCE exposure and cervical cancer

1

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al., 2005
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	Not reported		
	Medium cumulative TCE score			
	High TCE score			
	p for trend			
All employees at electronics factory (Taiwan)		0.96 (0.86, 1.22)[a]	337	Sung et al., 2007
Danish blue-collar worker w/TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure	1.9 (1.42, 2.37)	62	
	Exposure lag time			
	20 yrs	1.5 (0.7, 2.9)	9	
	Employment duration			
	<1 yr	2.5 (1.7, 3.5)	30	
	1−4.9 yrs	1.6 (1.0, 2.4)	22	
	≥5 yrs	1.3 (0.6, 2.4)	10	
Biologically-monitored Danish workers				Hansen et al., 2001
	Any TCE exposure	3.8 (1.0, 9.8)	4	
	Cumulative exposure (Ikeda)			
	<17 ppm-yr	2.9 (0.04, 16)	1	
	≥17 ppm-yr	2.6 (0.03, 14)	1	
	Mean concentration (Ikeda)			
	<4 ppm	3.4 (0.4, 12)	2	
	4+ ppm	4.3 (0.5, 16)	2	
	Employment duration			
	<6.25 yr	3.8 (0.1, 21)	1	
	≥6.25 yr	2.1 (0.03, 12)	1	
Aircraft maintenance workers from Hill Air Force Base, UT				Blair et al., 1998
	TCE subcohort	Not reported		
	Cumulative exposure	Not reported		
Biologically-monitored Finnish workers				Anttila et al., 1995
	All subjects	2.42 (1.05, 4.77)	8	
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	1.86 (0.38, 5.45)	3	
	6+ ppm	4.35 (1.41, 10.1)	5	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	Exposed workers	Not reported		

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Table 4-81. Summary of human studies on TCE exposure and cervical cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Biologically-monitored Swedish workers				Axelson et al., 1994
	Any TCE exposure	Not reported		
Cardboard manufacturing workers, Atlanta area, GA				Sinks et al., 1992
	All subjects	Not reported		
Cohort studies-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	Not reported		Boice et al., 2006
	Any exposure to TCE	Not reported		Zhao et al., 2005
View-Master employees				ATSDR, 2004
	Females	1.77 (0.57, 4.12)[b]	5	
United States uranium-processing workers (Fernald, OH)				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration	Not reported		
	Moderate TCE exposure, >2 yrs duration	Not reported		
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure	-- (0.00, 5.47)	0	
	Routine-intermittent	Not reported		
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	(0.00, 1.07)	0 (3.5 exp)	
	Low intensity (<50 ppm)		0 (1.91 exp)	
	High intensity (>50 ppm)		0 (1.54 exp)	
Aircraft maintenance workers (Hill AFB, UT)				Blair et al., 1998
	TCE subcohort	1.8 (0.5, 6.5)[c]	5	
	Cumulative exposure			
	0	1.0[c]		
	<5 ppm-yr	0.9 (0.1, 8.3)	1	
	5−25 ppm-yr		0	
	>25 ppm-yr	3.0 (0.8, 11.7)	4	
	TCE sucohort	1.67 (0.54, 5.22)	6	Radican et al., 2008
	Cumulative exposure			
	0	1.0[c]		
	<5 ppm-yr	0.76 (0.09, 6.35)	1	
	5-25 ppm-yr		0	
	>25 ppm-yr	2.83 (0.86, 9.33)	5	

Table 4-81. Summary of human studies on TCE exposure and cervical cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	TCE exposed workers	Not reported		
	Unexposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, MA)		Not examined[d]		Greenland et al., 1994
Cardboard manufacturing workers, Atlanta area, GA		Not reported		Sinks et al., 1992
U. S. Coast Guard employees		Not reported[e]		Blair et al., 1989
Aircraft manufacturing plant employees (Italy)		Not reported[e]		Costa et al., 1989
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects	0.61 (0.25, 1.26)[f]	7	
Lamp manufacturing workers (GE)				Shannon et al., 1988
	Coil/wire drawing	1.05 (0.03, 5.86)	1	
	Other areas	1.16 (0.32, 2.97)	4	
Case-control studies				
Geographic based studies				
Residents in two study areas in Endicott, NY		1.06 (0.29, 2.71)	<6	ATSDR, 2006
Residents in Texas				Coyle et al, 2005
	Counties reporting any air TCE release	66.4[g]		
	Countires not reporting any air TCE relesease	60.8 [g]		
Residents of 13 census tracts in Redlands, CA		0.65 (0.38, 1.02)	29	Morgan and Cassady, 2002
Finnish residents				
	Residents of Hausjarvi	Not reported		Vartiainen et al., 1993
	Residents of Huttula	Not reported		

[a]Standardized incidence ratio for females in Sung et al. (2007) reflects a 15-year lag period.
[b]Proportional mortality ratio.
[c]Internal referents, workers not exposed to TCE.
[d]Nested case-control analysis.
[e]Males only in cohort.
[f]SMR is for cancer of the genital organs (cervix, uterus, endometrium, etc.).
[g] Median annual age-adjusted breast cancer rate (1995-2000).

4.8.2.1.2. ***Breast cancer.*** Fifteen studies of TCE exposure reported findings on breast cancer in males and females combined (Garabrant et al., 1988; Greenland et al., 1994; Boice et al., 1999), in males and females, separately (Hansen et al., 2001; Raaschou-Nielsen et al., 2003; ATSDR, 2004; Clapp and Hoffman, 2008), or in females only (Shannon et al., 1988; Blair et al., 1998; Morgan et al., 1998; Coyle et al., 2005; ATSDR, 2006; Chang et al., 2005; Sung et al., 2007; Radican et al., 2008). Six studies have high likelihood of TCE exposure in individual

study subjects and met, to a sufficient degree, the standards of epidemiologic design and analysis (Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Radican et al. 2008). Four other high-quality studies with risk estimates for other cancer sites do not report risk estimates for breast cancer (Siemiatycki, 1991; Axelson et al., 1994; Anttila et al., 1995; Boice et al., 2006). No case-control studies were found on TCE exposure, although several studies examine occupational title or organic solvent as a class (Weiderpass et al., 1999; Band et al., 2000; Rennix et al., 2005; Ji et al., 2008). While association is seen with occupational title or industry and breast cancer (employment in aircraft and aircraft part industry, 2.48, 95% CI: 1.14, 5.39 [Band et al., 2000]; solvent user: 1.48, 95% CI: 1.03, 2.12 [Rennix et al., 2005]), TCE exposure is not uniquely identified. The two studies suggest association between organic solvents and female breast cancer needs further investigation of possible risk factors.

Relative risk estimates in the five high-quality studies ranged from 0.75 (0.43, 1.22) (females and males; Morgan et al., 1998) to 2.0 (0.9, 4.6) (mortality in females; Blair et al., 1998). Blair et al. (1998), additionally, observed stronger risk estimates for breast cancer mortality among females with low level intermittent (3.1, 95% CI: 1.5, 6.2) and low level continuous (3.4, 95% CI: 1.4, 8.0) TCE exposures, but not with frequent peaks (1.4, 95% CI: 0.7, 3.2). A similar pattern of risks was also observed by Radican et al. (2008) who studied mortality in this cohort and adding 10 years of follow-up, although the magnitude of breast cancer risk in females was lower than that observed in Blair et al. (1998). Risk estimates did not appear to increase with increasing cumulative exposure in the two studies that included exposure-response analyses (Blair et al., 1998; Morgan et al., 1998). None of the five high quality studies reported a statistically significant deficit in breast cancer and confidence intervals on relative risks estimates included 1.0 (no risk). Few female subjects in these studies appear to have high TCE exposure. For example, Blair et al. (1998) identified 8 of the 28 breast cancer deaths and 3 of the 34 breast cancer cases with high cumulative exposure.

Relative risk estimates in six studies of lower likelihood TCE exposure and other design deficiencies ranged from 0.81 (95% CI: 0.52, 1.48) (Garabrant et al., 1988) to 1.19 (1.03, 1.36) (Chang et al., 2005). These studies lack a quantitative surrogate for TCE exposure to individual subjects and instead classify all subjects as "potentially exposed", with resulting large dilution of actual risk and decreased sensitivity (Garabrant et al., 1988; Shannon et al., 1988; Morgan and Cassady, 2002; Chang et al., 2005; ATSDR, 2006; NRC, 2006; Sung et al., 2007).

Four studies reported on male breast cancer separately (Hansen et al., 2001; Raaschou-Nielsen et al., 2003; ATSDR, 2004; Clapp and Hoffman, 2008) and a total of three cases were observed. Breast cancer in men is a rare disease and is best studied using a case-control approach (Weiss et al., 2005). Reports exist of male breast cancer among former residents of

Camp Lejuene (U.S. EPA, 2009). Further assessment of TCE exposure and male breast cancer is warranted.

Overall, the epidemiologic studies on TCE exposure and breast cancer are quite limited in statistical power; observations are based on few breast cancer cases in high-quality studies or on inferior TCE exposure assessment in studies with large numbers of observed cases. Additionally, adjustment for nonoccupational breast cancer risk factors is less likely in cohort and geographic based studies given their use of employment and public records. Breast cancer mortality observations in Blair et al. (1998) and further follow-up of this cohort by Radican et al. (2008) of an elevated risk with overall TCE exposure, particulalry low level intermittent and continuous TCE exposure, provide evidence of an association with TCE. No other high-quality study reported a statistically significant association with breast cancer, although few observed cases leading to lower statistical power or examination of risk for males and females combined are alternative explanations for the null observations in these studies. Both Chang et al. (2005) and Sung et al. (2007), two overlapping studies of female electronics workers exposed to TCE, perchloroethylene, and mixed solvents, reported association with breast cancer incidence, with breast cancer risk in Chang et al. (2005) appearing to increase with employment duration. Both studies, in addition to association provided by studies of exposure to broader category of organic solvents (Band et al., 2000; Rennix et al., 2005), support Blair et al. (1998) and Radican et al. (2008), although the lack of exposure assessment is an uncertainty. The epidemiologic evidence is limited for examining TCE and breast cancer, and while these studies do not provide any strong evidence for association with TCE exposure they in turn do not provide evidence of an absence of association.

4.8.2.1.3. ***Cervical cancer.*** Eleven cohort or PMR studies and 2 geographic based studies present relative risk estimates (Garabrant et al., 1988; Shannon et al., 1988; Anttila et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999; Hansen et al., 2001; Morgan and Cassady, 2002; Raaschou-Nielsen et al., 2003; ATSDR, 2004, 2006; Sung et al., 2007; Radican et al., 2008). Seven of these studies had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Anttila et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Radican et al., 2008). Three small cohort studies (Costa et al., 1989; Sinks et al., 1992; Henschler et al., 1995) as well as three high-quality studies (Axelson et al., 1994; Zhao et al., 2005; Boice et al., 2006) did not present relative risk estimates for cervical cancer. Additionally, one population case-control and one geographic study of several site-specific cancers do not present information on cervical cancer (Siemiatycki, 1991; Vartiainen et al., 1993).

Five high-quality studies observed elevated risk for cervical cancer and overall TCE exposure (2.42, 95% CI: 1.05, 4.77 [Anttila et al., 1995]; 1.8, 95% CI: 0.5, 6.5 [Blair et al., 1998] that changed little with an additional 10 years follow-up, 1.67, 95% CI: 0.54, 5.22 [Radican et al., 2008]; 3.8, 95% CI: 1.42, 2.37 [Hansen et al., 2001]; 1.9, 95% CI: 1.42, 2.37 [Raaschou-Nielsen et al., 2003]). The observations of a 3- to 4-fold elevated cervical cancer risk with high mean TCE exposure compared to subjects in the low exposure category (6+ ppm: 4.35, 95% CI: 1.41, 10.1 [Anttila et al., 1995]; 4+ ppm: 4.3, 95% CI: 0.5, 16 [Hansen et al., 2001]) or with high cumulative TCE exposure (0.25-ppm year: 3.0, 95% CI: 0.8, 11.7 [Blair et al., 1998], 2.83, 95% CI: 0.86, 9.33 [Radican et al., 2008]) provides additional support for association with TCE. Cervical cancer risk was lowest for subjects in the high exposure duration category (Hansen et al., 2001; Raaschou-Nielsen et al., 2003); however, duration of employment is a poor exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006). No deaths due to cervical cancer were observed in two other high-quality studies (Morgan et al., 1998; Boice et al., 1999), less than 4 deaths were expected, suggesting these cohorts contained few female subjects with TCE exposure.

Human papilloma virus and low socioeconomic status are known risk factors for cervical cancer (ACS, 2008). Subjects in Raaschou-Nielsen et al. (2003) are blue-collar workers and low socioeconomic status likely explains observed associations in this and the other high-quality studies. The use of internal controls in Blair et al. (1998) who are similar in socioeconomic status as TCE subjects is believed to partly account for possible confounder related to socio-economic status; however, direct information on individual subjects is lacking.

Six other cohort, PMR, and geographic based studies were given less weight in the analysis because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity (Garabrant et al., 1988; Shannon et al., 1988; Morgan and Cassady, 2002; ATSDR, 2004, 2006; Sung et al., 2007). Cervical cancer risk estimates in these studies ranged between 0.65 (95% CI: 0.38, 1.02) (Morgan and Cassady, 2002) to 1.77 (proportional mortality ratio; 95% CI: 0.57, 4.12; ATSDR, 2004). No study reported a statistically significant deficit in cervical cancer risk.

4.8.2.2. *Animal studies*

Histopathology findings have been noted in reproductive organs in various cancer bioassay studies conducted with TCE. A number of these findings (summarized in Table 4-82) do not demonstrate a treatment-related profile.

Table 4-82. Histopathology findings in reproductive organs

Tumor incidence in mice after 18 months inhalation exposure [a]								
	Tissue	**Finding**	**Control**	**100 ppm**	**500 ppm**			
Males	No. examined:		30	29	30			
	Prostate	Myoma	1	0	0			
	Testis	Carcinoma	0	0	1			
		Cyst	0	0	1			
Females	No. examined:		29	30	28			
	Uterus	Adenocarcinoma	1	0	0			
	Ovary	Adenocarcinoma	1	0	0			
		Adenoma	3	1	3			
		Carcinoma	0	2	2			
		Granulosa cell tumor	4	0	2			
Tumor incidence in rats after 18 months inhalation exposure [a]								
	Tissue	**Finding**	**Control**	**100 ppm**	**500 ppm**			
Males	No. examined:		29	30	30			
	Testis	Interstitial cell tumors	4	0	3			
Females	No. examined:		28	30	30			
	Mammary	Fibroadenoma	2	0	0			
		Adenocarcinoma	3	2	2			
	Uterus	Adenocarcinoma	3	1	4			
	Ovary	Carcinoma	4	0	1			
		Granulosa cell tumor	1	0	0			
	Genital tract	Squamous cell carcinoma	0	2	0			
Tumor incidence in hamsters after 18 months inhalation exposure [a]								
	Tissue	**Finding**	**Control**	**100 ppm**	**500 ppm**			
Females	No. examined:		30	29	30			
	Ovary	Cystadenoma	1	0	0			
Tumor incidence in mice after 18 months gavage administration [b]								
	Tissue	**Finding**	**Con-trol**	**TCE Pure**	**TCE Industrial**	**TCE+ EPC**	**TCE +BO**	**TCE +EPC +BO**
Females	No. examined:		50	50	50	50	48	50
	Mammary	Carcinoma	1	2	0	0	0	0
	Ovary	Granulosa cell tumor	0	1	0	0	0	0
	Vulva	Squamous cell carcinoma	0	0	0	0	1	1

[a]Henschler et al. (1980).
[b]Henschler et al. (1984); EPC = epichlorohydrin; BO = 1,2-epoxybutane.

Cancers of the reproductive system that are associated with TCE exposure and observed in animal studies are comprised of testicular tumors (interstitial cell and Leydig cell) (U.S. EPA, 2001). A summary of the incidences of testicular tumors observed in male rats is presented in Table 4-83.

Table 4-83. Testicular tumors in male rats exposed to TCE, adjusted for reduced survival[a]

Interstitial cell tumors after 103 wks gavage exposure, beginning at 6.5−8 wks of age (NTP, 1988, 1990)				
Administered dose (mg/kg/d)	**Untreated control**	**Vehicle control**	**500**	**1,000**
Male ACI rats	38/45 (84%)	36/44 (82%)	23/26 (88%)	17/19 (89%)
Male August rats	36/46 (78%)	34/46 (74%)	30/34 (88%)	26/30 (87%)
Male Marshall rats[b]	16/46 (35%)	17/46 (37%)	21/33 (64%)	32/39 (82%)
Male Osborne-Mendel rats	1/30 (3%)	0/28 (0%)	0/25 (0%)	1/19 (5%)
Male F344/N rats	44/47 (94%)	47/48 (98%)	47/48 (98%)	32/44 (73%)
Leydig cell tumors after 104 wks inhalation exposure, beginning at 12 wks of age (Maltoni et al., 1986)				
Administered daily concentration (mg/m^3)[c]	**Control**	**112.5**	**337.5**	**675**
Male Sprague-Dawley rats[b]	6/114 (5%)	16/105 (15%)	30/107 (28%)	31/113 (27%)

[a]ACI rats alive at Week 70, August rats at Week 65, Marshall rats at Week 32, Osborne-Mendel rats at Week 97, F344/N rats at Week 32, Sprague-Dawley rats at Week 81 (except BT304) or Week 62 (except BT304 bis).
[b]Equivalent to 100, 300, or 600 ppm (100 ppm = 540 mg/m^3), adjusted for 7 hours/day, 5 days/week exposure.
[c]Statistically significant by Cochran-Armitage trend test ($p < 0.05$).

Sources: NTP (1988) Tables A2, C2, E2, G2; NTP (1990) Table A3; Maltoni et al. (1986) IV/IV Table 21, IV/V Table 21.

4.8.2.3. *Mode of Action for Testicular Tumors*

The database for TCE does not include an extensive characterization of the mode of action for Leydig cell tumorigenesis in the rat, although data exist that are suggestive of hormonal disruption in male rats. A study by Kumar et al. (2000b) found significant decreases in serum testosterone concentration and in 17-β-HSD, G6PDH, and total cholesterol and ascorbic acid levels in testicular homogenate from male rats that had been exposed via inhalation to 376 ppm TCE for 12 or 24 weeks. In a follow-up study, Kumar et al. (2001) also identified decreases in SDH in the testes of TCE-treated rats. These changes are markers of disruption to testosterone biosynthesis. Evidence of testicular atrophy, observed in the 2001 study by

Kumar et al., as well as the multiple *in vivo* and *in vitro* studies that observed alterations in spermatogenesis and/or sperm function, could also be consistent with alterations in testosterone levels. Therefore, while the available data are suggestive of a MOA involving hormonal disruption for TCE-induced testicular tumors, the evidence is inadequate to specify and test a hypothesized sequence of key events.

Leydig cell tumors can be chemically induced by alterations of steroid hormone levels, through mechanisms such as agonism of estrogen, gonadotropin releasing hormone, or dopamine receptors; antagonism of androgen receptors; and inhibition of 5α-reductase, testosterone biosynthesis, or aromatase (Cook et al., 1999). For those plausible mechanisms that involve disruption of the hypothalamic-pituitary-testis (HPT) axis, decreased testosterone or estradiol levels or recognition is involved, and increased luteinizing hormone (LH) levels are commonly observed. Although there is evidence to suggest that humans are quantitatively less sensitive than rats in their proliferative response to LH, evidence of treatment-related Leydig cell tumors in rats that are induced via HPT disruption is considered to represent a potential risk to humans (with the possible exception of GnRh or dopamine agonists), since the pathways for regulation of the HPT axis are similar in rats and humans (Clegg et al., 1997).

4.8.3. Developmental Toxicity

An evaluation of the human and experimental animal data for developmental toxicity, considering the overall weight and strength of the evidence, suggests a potential for adverse outcomes associated with pre- and/or postnatal TCE exposures.

4.8.3.1. *Human Developmental Data*

Epidemiological developmental studies (summarized in Table 4-84) examined the relationship between TCE exposure and prenatal developmental outcomes including spontaneous abortion and perinatal death; decreased birth weight, small for gestational age, and postnatal growth; congenital malformations; and other adverse birth outcomes. Postnatal developmental outcomes examined include developmental neurotoxicity, developmental immunotoxicity, other developmental outcomes, and childhood cancer.

Table 4-84. Developmental studies in humans

Subjects	Exposure	Effect	Reference
Adverse fetal/birth outcomes			
Spontaneous abortion and perinatal death			
371 men occupationally exposed to solvents in Finland 1973−1983	Questionnaire Low/rare used <1 d/wk; Intermediate used 1−4 d/wk or intermediate/low TCA urine levels; High/frequent used daily or high TCA urine levels	No risk of spontaneous abortion after paternal exposure, based on 17 cases and 35 controls exposed to TCE (OR: 1.0, 95% CI: 0.6−2.0)	Taskinen et al., 1989
535 women occupationally exposed to solvents in Finland 1973−1986	Questionnaire Rare used 1−2 d/wk; Frequent used ≥3 d/wk	Increased risk of spontaneous abortion among frequently-exposed women, based on 7 cases and 9 controls exposed to TCE (OR: 1.6, 95% CI: 0.5−4.8)	Taskinen et al., 1994
3,265 women occupationally exposed to organic solvents in Finland 1973−1983	Questionnaire U-TCA: median: 48.1 µmol/L; mean 96.2 ± 19.2 µmol/L	No increased risk of spontaneous abortion based on 3 cases and 13 controls exposed to TCE OR: 0.6, 95% CI: 0.2−2.3	Lindbohm et al., 1990
361 women occupationally and residentially exposed to solvents in Santa Clara County, CA June 1986−February 1987 (735 controls)	Questionnaire	Increased risk of spontaneous abortion based on 6 cases and 4 controls exposed to TCE[a] OR: 3.1, 95% CI: 0.92−10.4	Windham et al., 1991
4,396 pregnancies among residents of Woburn, MA 1960−1982	TCE: 267 µg/L Tetrachloroethylene: 21 µg/L Chloroform: 12 µg/L	Increased risk of perinatal death (n = 67) after 1970 (p = 0.55) but not before 1970 (OR: 10, p = 0.003) No increased risk of spontaneous abortion (n = 520; p = 0.66)	Lagakos et al., 1986
707 parents of children with congenital heart disease in Tucson Valley, AZ 1969−1987	6−239 ppb TCE, along with DCA and chromium	No increased risk of fetal death (not quantified) based on 246 exposed and 461 unexposed cases	Goldberg et al., 1990
75 men and 71 women living near Rocky Mountain Arsenal, CO 1981−1986	Low: <5.0 ppb Medium: ≥5.0 to <10.0 ppb High: <10.0 ppb	Increased risk of miscarriage OR_{adj}: 4.44, 95% CI: 0.76−26.12 Increased risk of no live birth OR_{adj}: 2.46, 95% CI: 0.24−24.95	ATSDR, 2001
1,440 pregnancies among residents of Endicott, NY 1978−2002	indoor air from soil vapor: 0.18−140 mg/m^3	No increase in spontaneous fetal death SIR: 0.66, 95% CI: 0.22−1.55	ATSDR, 2006, 2008

Table 4-84. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
81,532 pregnancies among residents of 75 New Jersey towns 1985−1988 (3 control groups)	55 ppb TCE, along with many other compounds	No increased risk of fetal death for >10 ppb OR: 1.12	Bove, 1996; Bove et al., 1995
Decreased birth weight, small for gestational age, and postnatal growth			
361 women occupationally and residentially exposed to solvents in Santa Clara County, CA June 1986−February 1987 (735 controls)	Questionnaire	Increased risk of IUGR based on one case exposed to both TCE and tetrachloroethylene OR: 12.5	Windham et al., 1991
3,462 births in Woburn, MA 1960−1982	267 µg/L TCE in drinking water, along with tetrachloroethylene and chloroform	No increase in low birth weight (p = 0.77)	Lagakos et al., 1986
1,099 singleton births[b] to residents of 3 census tracts near Tucson International Airport 1979−1981 (877 controls)	<5−107 µg/L	No increase in full-term low birth weight (OR: 0.81) No increase in low birth weight (OR: 0.9) Increase in very low birth weight OR: 3.3, 95% CI: 0.53−20.6	Rodenbeck et al., 2000
1,440 births[c] to residents of Endicott, NY 1978−2002	Indoor air from soil vapor: 0.18−140 mg/m^3	Small increase in low birth weight OR: 1.26, 95% CI: 1.00−1.59 Small increase in small for gestational age OR: 1.22, 95% CI: 1.02−1.45 Increase in full-term low birth weight OR: 1.41, 95% CI: 1.01−1.95	ATSDR, 2006, 2008
6,289 pregnancies among women residing at Camp Lejeune, NC 1968−1985 (141 short-term and 31 long-term TCE-exposed, 5,681 unexposed controls)[d]	Tarrawa Terrace: TCE: 8 ppb; 1,2-DCE: 12 ppb PCE: 215 ppb Hadnot Point: TCE: 1,400 ppb 1,2-DCE: 407 ppb	Change in mean birth weight Long-term total: -139 g, 90% CI: -277, -1 Long-term males: -312 g, 90% CI: -540, -85 Short term total: +70g, 90% CI: -6, 146 Increase in SGA Long-term total: OR: 1.5, 90% CI: 0.5, 3.8 Long-term males: OR: 3.9, 90% CI: 1.1−11.9 Short term total: OR: 1.1, 90% CI: 0.2−1.1	ATSDR, 1998

Table 4-84. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
81,532 pregnancies[c] among residents of 75 New Jersey towns 1985−1988	55 ppb TCE, along with many other compounds	Decreased birth weight at >5 ppb by 17.9g No increase in prematurity at >10 ppb: OR: 1.02 Increase in low birth weight, term >10 ppb: OR: 1.23, 50% CI: 1.09−1.39 No risk for very low birth weight	Bove, 1996; Bove et al., 1995
Congenital malformations			
1,148 men and 969 women occupationally exposed to TCE in Finland 1963−1976	U-TCA: <10 to >500 mg/L	No congenital malformations reported	Tola et al., 1980
371 men occupationally exposed to solvents in Finland 1973−1983	Low/rare used <1 d/wk; Intermediate used 1−4 d/wk or if biological measures indicated high exposure; High/frequent used daily or if biological measures indicated high exposure	No increase in congenital malformations based on 17 cases and 35 controls exposed to TCE OR: 0.6, 95% CI: 0.2−2.0	Taskinen et al., 1989
100 babies with oral cleft defects born to women occupationally exposed in Europe 1989−1992	Questionnaire	Increase in cleft lip based on 2 of 4 TCE-exposed women ORa_{dj}: 3.21, 95% CI: 0.49−20.9 Increase in cleft palate based on 2 of 4 TCE-exposed women ORa_{dj}: 4.47, 95% CI: 1.02−40.9	Lorente et al., 2000
4,396 pregnancies among residents of Woburn, MA 1960−1982	TCE: 267 µg/L Tetrachloroethylene: 21 µg/L Chloroform: 12 µg/L	Increase in eye/ear birth anomalies: OR: 14.9, $p < 0.0001$ Increase in CNS/chromosomal/oral cleft anomalies: OR: 4.5, $p = 0.01$ Increase in kidney/urinary tract disorders: OR: 1.35, $p = 0.02$ Small increase in lung/respiratory tract disorders: OR: 1.16, $p = 0.05$ No increase in cardiovascular anomalies ($n = 5$): $p = 0.91$	Lagakos et al., 1986
707 children with congenital heart disease in Tucson Valley, AZ 1969−1987 (246 exposed, 461 unexposed)	Wells contaminated with TCE (range: 6−239 ppb), along with DCA and chromium	Increase in congenital heart disease <1981: OR: ≈3 ($p < 0.005$) >1981: OR: ≈1 Increased prevalence after maternal exposure during first trimester ($p < 0.001$, 95% CI: 1.14−4.14)	Goldberg et al., 1990

Table 4-84. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
75 men, 71 women living near Rocky Mountain Arsenal, CO 1981−1986	Low: <5.0 ppb Medium: ≥5.0 to <10.0 ppb High: <10.0 ppb	Increase in total birth defects (n = 9) OR: 5.87, 95% CI: 0.59−58.81	ATSDR, 2001
Births to residents of Endicott, NY 1983−2000[f]	Indoor air from soil vapor: 0.18−140 mg/m^3	No increase in total birth defects: RR: 1.08, 95% CI: 0.82−1.42 Increase in total cardiac defects: RR: 1.94, 95% CI: 1.21−3.12 Increase in major cardiac defects: RR: 2.52, 95% CI: 1.2−5.29 Increase in conotruncal heart defects: RR: 4.83, 95% CI: 1.81−12.89	ATSDR, 2006, 2008
81,532 pregnancies among residents of 75 New Jersey towns 1985−1988	55 ppb TCE, along with many other compounds	No increase in total birth defects: >10 ppb: OR: 1.12 Increase in total CNS defects at high dose >1−5 ppb: OR: 0.93, 90% CI: 0.47−1.77 >10 ppb: OR: 1.68, 90% CI: 0.76−3.52 Increase in neural tube defects >1−5 ppb: OR: 1.58, 90% CI: 0.69−3.40 >10 ppb: OR: 2.53, 90% CI: 0.91−6.37 Increase in oral clefts: >5 ppb: OR: 2.24, 95% CI: 1.16−4.20 Increase in major cardiac defects: >10 ppb: OR: 1.24, 50% CI: 0.75−1.94 Increase in ventrical septal defects >5ppb: OR: 1.30, 95% CI: 0.88−1.87	Bove, 1996; Bove et al., 1995
1,623 children <20 yrs old dying from congenital anomalies in Maricopa County, AZ 1966−1986	8.9 and 29 ppb TCE in drinking water	Increase in deaths due to congenital anomalies in East Central Phoenix 1966−1969: RR: 1.4, 95% CI: 1.1−1.7 1970−1981: RR: 1.5, 95% CI: 1.3−1.7 1982−1986: RR: 2.0, 95% CI: 1.5−2.5	AZ DHS, 1988
4,025 infants born with congenital heart defects in Milwaukee, WI 1997−1999	Maternal residence within 1.32 miles from at least one TCE emissions source	Increase in congenital heart defects for mothers ≥38 yrs old Exposed: OR: 6.2, 95% CI: 2.6−14.5 Unexposed: OR: 1.9, 95% CI: 1.1−3.5 No increase in congenital heart defects for exposed mothers <38 yrs old: OR: 0.9, 95% CI: 0.6−1.2	Yauck et al., 2004

Table 4-84. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
12 children exposed to TCE in well water in Michigan	5−10 yrs to 8−14 ppm	1 born with multiple birth defects	Bernad et al., 1987, abstract
Other adverse birth outcomes			
34 live births for which inhalation of TCE for anesthesia was used in Japan 1962−1697	2−8 mL (mean 4.3 mL) for 2−98 min (mean: 34.7 min)	1 case of asphyxia; 3 "sleepy babies" with Apgar scores of 5−9. Delayed appearance of newborn reflexes	Beppu, 1968
51 UK women whose fetus was considered to be at risk for hypoxia during labor administered TCE as an analgesic (50 controls)	Amount and route of exposure not reported	TCE caused fetal pH to fall more, base deficit increased more, and PO_2 fell more than the control group by 4-fold or more compared to other analgesics used	Phillips and Macdonald, 1971
Postnatal developmental outcomes			
Developmental neurotoxicity			
54 individuals from 3 residential cohorts in the United States exposed to TCE in drinking water	Woburn, MA 63−400 ppb for <1−12 yrs Alpha, OH 3.3−330 ppb for 5−17 yrs Twin Cities, MN 261−2,440 ppb for 0.25−25 yrs	Woburn, MA Verbal naming/language impairment in 6/13 children (46%) Alpha, OH Verbal naming/language impairment in 1/2 children (50%) Twin Cities, MN Verbal naming/language impairment in 4/4 children (100%) Memory impairment in 4/4 children (100%) Academic impairment in 4/4 children (100%) Moderate encephalopathy in 4/4 children (100%) Poor performance on reading/spelling test in 3/4 children (75%) Poor performance on information test in 3/4 children (75%)	White et al., 1997
284 cases of ASD diagnosed <9 yrs old and 657 controls born in the San Francisco Bay Area 1994	Births geocoded to census tracts, and linked to HAPs data	Increase in ASD upper 3rd quartile: OR: 1.37, 95% CI: 0.96−1.95 upper 4th quartile: OR: 1.47, 95% CI: 1.03−2.08	Windham et al., 2006
948 children (<18 yrs) in the trichloroethylene Subregistry	0.4 to >5,000 ppb TCE	Increase in speech impairment: 0−9 yrs old: RR: 2.45, 99% CI: 1.31−4.58 10−17 yrs old: RR: 1.14, 99% CI: 0.46−2.85 Increase in hearing impairment: 0−9 yrs old: RR: 2.13, 99% CI: 1.12−4.07 10−17 yrs old: RR: 1.12, 99% CI: 0.52−2.24	ATSDR, 2003a; Burg et al., 1995; Burg and Gist, 1999

Table 4-84. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
12 children exposed to TCE in well water in Michigan	5−10 yrs to 8−14 ppm	9 of 12 children (75%) had poor learning ability, aggressive behavior, and low attention span	Bernad et al., 1987, abstract
Developmental immunotoxicity			
200 children aged 36 months old born prematurely[g] and at risk of atopy[h] in Lepzig, Germany 1995−1996	Median air level in child's bedroom: 0.42 μg/m^3	No association with allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells	Lehmann et al., 2001
85 healthy[i] full-term neonates born in Lepzig, Germany 1997−1999	Median air level in child's bedroom 3−4 wks after birth: 0.6 μg/m^3	Significant reduction of Th1 IL-2 producing T-cells	Lehmann et al., 2002
Other developmental outcomes			
55 children (6 months to 10 yrs old) were anesthetized for operations to repair developmental defects of the jaw and face in Poland 1964	≥10 mL TCE	Reports of bradycardia, accelerated heart rate, and respiratory acceleration observed; no arrhythmia was observed	Jasinka, 1965, translation
Childhood cancer			
98 children (<10 yrs old) diagnosed with brain tumors in Los Angeles County 1972−1977	Questionnaire of parental occupational exposures	Two cases were reported for TCE exposure, one with methyl ethyl ketone	Peters et al., 1981
22 children (<19 yrs old) diagnosed with neuroblastoma in United States and Canada 1992−1994 (12 controls)	Questionnaire of parental occupational exposures	Increase in neuroblastoma after paternal exposure OR: 1.4, 95% CI: 0.7−2.9 Maternal exposure not reported	De Roos et al., 2001
61 boys and 62 girls (<10 yrs old) diagnosed with leukemia and 123 controls in Los Angeles County 1980−1984	Questionnaire of parents for occupational exposure	Increase in leukemia after paternal exposure Preconception (1 yr): OR: 2.0, $p = 0.16$ Prenatal: OR: 2.0, $p = 0.16$ Postnatal: OR: 2.7, $p = 0.7$ Maternal exposure not reported	Lowengart et al., 1987
1,842 children (<15 yrs old) diagnosed with ALL in United States and Canada 1989−1993 (1986 controls)	Questionnaire of parents for occupational exposure	Increase in ALL after maternal exposure Preconception: OR: 1.8, 95% CI: 0.6−5.2 Pregnancy: OR: 1.8, 95% CI: 0.5−6.4 Postnatal: OR: 1.4, 95% CI: 0.5−4.1 Anytime: OR: 1.8, 95% CI: 0.8−4.1 No increase in ALL after paternal exposure Anytime: OR: 1.1, 95% CI: 0.8−1.5	Shu et al., 1999

Table 4-84. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
109 children (<15 yrs old) born in UK 1974−1988 (218 controls)	Questionnaire of parents for occupational exposure	Increase in leukemia and NHL after paternal exposure Preconception: OR: 2.27, 95% CI: 0.84−6.16 Prenatal: OR: 4.40, 95% CI: 1.15−21.01 Postnatal: OR: 2.66, 95% CI: 0.82−9.19 No increase in leukemia and NHL after maternal exposure Preconception: OR: 1.16, 95% CI: 0.13−7.91	McKinney et al., 1991
22 children (<15 yrs old) diagnosed with childhood cancer in California 1988−1998	0.09−97 ppb TCE in drinking water	No increase in total cancer: SIR: 0.83, 99% CI: 0.44−1.40 No increase in CNS cancer: SIR: 1.05, 99% CI: 0.24−2.70 No increase in leukemia: SIR: 1.09, 99% CI: 0.38−2.31	Morgan and Cassady, 2002
1,190 children (<20 yrs old) diagnosed with leukemia in 4 counties in New Jersey 1979−1987	0−67 ppb TCE in drinking water	Increase in ALL in girls with >5 ppb exposure <20 yrs old: RR: 3.36, 95% CI: 1.29−8.28 <5 yrs old: RR: 4.54, 95% CI: 1.47−10.6	Cohn et al., 1994
24 children (<15 yrs old) diagnosed with leukemia in Woburn, MA 1969−1997	267 µg/L TCE in drinking water, along with tetrachloroethylene, arsenic, and chloroform	Increase in childhood leukemia Preconception: OR_{adj}: 2.61, 95% CI: 0.47−14.97 Pregnancy: OR_{adj}: 8.33, 95% CI: 0.73−94.67 Postnatal: OR_{adj}: 1.18, 95% CI: 0.28−5.05 Ever: OR_{adj}: 2.39, 95% CI: 0.54−10.59	Costas et al., 2002; Cutler et al., 1986; Lagakos et al., 1986; MA DPH, 1997[j]
347 children (<20 yrs old) diagnosed with cancer in Endicott, NY 1980−2001	indoor air from soil vapor: 0.18−140 mg/m^3	No increase in cancer (<6 cases, similar to expected)	ATSDR, 2006, 2008
189 children (<20 yrs old) diagnosed with cancer in Maricopa County, AZ 1965−1990	8.9 and 29 ppb TCE in drinking water	Increase in leukemia: 1965−1986: SIR: 1.67, 95% CI: 1.20−2.27 1982−1986: SIR: 1.91, 95% CI: 1.11−3.12	AZ DHS, 1988, 1990a, 1997[k]
		No increase in total childhood cancers, lymphoma, brain/CNS, or other cancers	
16 children (<20 yrs old) diagnosed with cancer in East Phoenix, AZ 1965−1986	TCE, TCA, and other contaminants in drinking water	No increase in leukemia: SIR: 0.85, 95% CI: 0.50−1.35	AZ DHS, 1990b

Table 4-84. Developmental studies in humans (continued)

Subjects	Exposure	Effect	Reference
37 children (<20 yrs old) diagnosed with cancer in Pima County, AZ 1970−1986	1.1−239 ppb TCE, along with 1,1-DCE, chloroform and chromium in drinking water	Increase in leukemia (*n* = 11): SIR: 1.50, 95% CI: 0.76−2.70 No increase in testicular cancer (*n* = 6): SIR: 0.78, 95% CI: 0.32−1.59 No increase in lymphoma (*n* = 2): SIR: 0.63, 95% CI: 0.13−1.80 No increase in CNS/brain cancer (*n* = 3): SIR: 0.84, 95% CI: 0.23−2.16 Increase in other cancer (*n* = 15): SIR: 1.40, 95% CI: 0.79−2.30	AZ DHS, 1990c

[a]Of those exposed to TCE, four were also exposed to tetrachloroethylene and one was also exposed to paint strippers and thinners.
[b]Full term defined as between 35 and 46 weeks gestation, low birth weight as <2501 g, and very low birth weight as <1,501 g.
[c]Low birth weight defined as <2,500, moderately low birth weight (1,500−<2,500 g), term low birth weight (≥37 weeks gestation and <25,000 g).
[d]Unexposed residents resided at locations not classified for long-term or short-term TCE exposure. Long-term TCE exposed mothers resided at Hospital Point during 1968−1985 for at least one week prior to birth. Short-term TCE exposed mothers resided at Berkeley Manor, Midway Park, Paradise Point, and Wakins Village at the time of birth and at least 1 week during January 27 to February 7, 1985. In addition, the mother's last menstrual period occurred on or before January 31, 1985 and the birth occurred after February 2, 1985.
[e]Low birth weight defined as <2,500 g, very low birth weight as <1,500 g.
[f]1,440 births reported for years 1978−2002, but number not reported for years 1983−2000.
[g]Premature defined as 1,500−2,500 g at birth.
[h]Risk of atopy defined as cord blood IgE >0.9 kU/L; double positive family atopy history.
[i]Healthy birth defined as ≥2,500 g and ≥37 weeks gestation.
[j]Only results from Costas et al. (2002) are reported in the table.
[k]Only results from AZ DHS (1990a) are reported in the table.

PCE = perchloroethylene, UK = United Kingdom.

4.8.3.1.1. ***Adverse fetal/birth outcomes.***

4.8.3.1.1.1. ***Spontaneous abortion and perinatal death.*** Spontaneous abortion or miscarriage is defined as nonmedically induced premature delivery of a fetus prior to 20 weeks gestation. Perinatal death is defined as stillbirths and deaths before 7 days after birth. Available data comes from several studies of occupational exposures in Finland and Santa Clara, California, and by geographic-based studies in areas with known contamination of water supplies in Woburn, MA; Tucson Valley, AZ; Rocky Mountain Arsenal, CO; Endicott, NY; and New Jersey.

4.8.3.1.1.1.1. *Occupational studies*. The risks of spontaneous abortion and congenial malformations among offspring of men occupationally exposed to TCE and other organic solvents were examined by Taskinen et al. (1989). This nested case-control study was conducted in Finland from 1973−1983. Exposure was determined by biological measurements of the father and questionnaires answered by both the mother and father. The level of exposure was classified as "low/rare" if the chemical was used <1 days/week, "intermediate" if used 1−4 days/week or if TCA urine measurements indicated intermediate/low exposure, and "high/frequent" if used daily or if TCA urine measurements indicated clear occupational exposure (defined as above the RfV for the general population). There was no risk of spontaneous abortion from paternal TCE exposure (OR: 1.0, 95% CI: 0.6−2.0), although there was a significant increase for paternal organic solvent exposure (OR: 2.7, 95% CI: 1.3−5.6) and a nonsignificant increase for maternal organic solvent exposure (OR: 1.4, 95% CI: 0.6−3.0). (Also see section below for results from this study for congenital malformations).

Another case-control study in Finland examined pregnancy outcomes in 1973−1986 among female laboratory technicians aged 20−34 years (Taskinen et al., 1994). Exposure was reported via questionnaire, and was classified as "rare" if the chemical was used 1−2 days/week, and "frequent" if used at least 3 days/week. Cases of spontaneous abortion (n = 206) were compared with controls who had delivered a baby and did not report prior spontaneous abortions (n = 329). A nonstatistically significant increased risk was seen between spontaneous abortion and TCE use at least 3-days-a-week (OR: 1.6, 95% CI: 0.5−4.8).

The association between maternal exposure to organic solvents and spontaneous abortion was examined in Finland for births 1973−1983 (Lindbohm et al., 1990). Exposure was assessed by questionnaire and confirmed with employment records, and the level of exposure was either high, low or none based on the frequency of use and known information about typical levels of exposure for job type. Biological measurements of trichloroacetic acid in urine were also taken on 64 women, with a median value of 48.1 μmol/L (mean: 96.2 ± 19.2 μmol/L). Three cases and 13 controls were exposed to TCE, with no increased risk seen for spontaneous abortion (OR: 0.6, 95% CI: 0.2−2.3, p. 0.45).

A case-control study in Santa Clara County, California, examined the association between solvents and adverse pregnancy outcomes in women ≥18 years old (Windham et al., 1991). For pregnancies occurring between June 1986 and February 1987, 361 cases of spontaneous abortion were compared to 735 women who had a live birth during this time period. Telephone interviews included detailed questions on occupational solvent exposure, as well as additional questions on residential solvent use. For TCE exposure, six cases of spontaneous abortion were compared to four controls of live births; of these ten TCE-exposed individuals, four reported exposure to tetrachloroethylene, and one reported exposure to paint strippers and thinners. An increased risk of spontaneous abortions was seen with TCE exposure (OR: 3.1, 95% CI: 0.92−10.4), with a statistically significant increased risk for those exposed ≥0.5 hours/week (OR: 7.7, 95% CI: 1.3−47.4). An increased risk for spontaneous abortion was also seen for those reporting a more "intense" exposure based primarily on odor, as well as skin contact or other symptoms (OR: 3.9, $p = 0.04$). (Also see section below from this study on low birth weight.)

4.8.3.1.1.1.2. *Geographic-based studies*. A community in Woburn, MA with contaminated well water experienced an increased incidence of adverse birth outcomes and childhood leukemia (Lagakos et al., 1986). In 1979, the wells supplying drinking water were found to be contaminated with 267 ppb TCE, 21 ppb tetrachloroethylene, 11.8 ppb, and 12 ppb chloroform, and were subsequently closed. Pregnancy and childhood outcomes were examined from 4,396 pregnancies among residents (Lagakos et al., 1986). No association between water access and incidence of spontaneous abortion ($n = 520$) was observed ($p = 0.66$). The town's water distribution system was divided into five zones, which was reorganized in 1970. Prior to 1970, no association was observed between water access and incidence of perinatal deaths ($n = 46$ still births and 21 deaths before 7 days) ($p = 0.55$). However, after 1970, a statistically significant positive association between access to contaminated water and perinatal deaths was observed (OR: 10.0, $p = 0.003$). The authors could not explain why this discrepancy was observed, but speculated that contaminants were either not present prior to 1970, or were increased after 1970. (Also see sections below on decreased birth weight, congenital malformations, and childhood cancer for additional results from this cohort.)

A community in Tucson Valley, Arizona with contaminated well water had a number of reported cases of congenital heart disease. The wells were found to be contaminated with TCE (range = 6−239 ppb), along with dichloroethylene and chromium (Goldberg et al., 1990). This study identified 707 children born with congenital heart disease during the years 1969−1987. Of the study participants, 246 families had parental residential and occupational exposure during one month prior to conception and during the first trimester of pregnancy, and 461 families had

no exposure before the end of the first trimester. In addition to this control group, two others were used: (1) those that had contact with the contaminated water area, and (2) those that had contact with the contaminated water area and matched with cases for education, ethnicity, and occupation. Among these cases of congenital heart disease, no significant difference was seen for fetal death (not quantified) for exposed cases compared to unexposed cases. (Also see section below on congenital malformations for additional results from this cohort.)

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the outcomes in offspring of 75 men and 71 women exposed to TCE in drinking water (ATSDR, 2001). TCE exposure was stratified by high (>10.0 ppb), medium (≥5.0 ppm to <10.0 ppb), and low (<5.0 ppb). Among women with >5 ppb exposure experiencing miscarriage (n = 22/57) compared to unexposed women experiencing miscarriage (n = 2/13) an elevated nonsignificant association was observed (OR_{adj}: 4.44, 95% CI: 0.76−26.12). For lifetime number of miscarriages reported by men and women, results were increased but without dose-response for women (medium: OR_{adj}: 8.56, 95% CI: 0.69−105.99; high: OR_{adj}: 4.16, 95% CI: 0.61−25.99), but less for men (medium: OR_{adj}: 1.68, 95% CI: 0.26−10.77; high: OR_{adj}: 0.65, 95% CI: 0.12−3.48). Among women with >5 ppb exposure experiencing no live birth (n = 9/57) compared to unexposed women experiencing no live birth (n = 1/13) an elevated nonsignificant association was observed (OR_{adj}: 2.46, 95% CI: 0.24−24.95). (Also see below for results from this study on birth defects.)

NYS DOH and ATSDR conducted a study in Endicott, NY to examine childhood cancer and birth outcomes in an area contaminated by a number of volatile organic compounds (VOCs), including "thousands of gallons" of TCE (ATSDR, 2006). Soil vapor levels tested ranged from 0.18−140 mg/m^3 in indoor air. A follow-up study by ATSDR (2008) reported that during the years 1978−1993 only five spontaneous fetal deaths occurring ≥20 weeks gestation were reported when 7.5 were expected (SIR: 0.66, 95% CI: 0.22−1.55). (See sections on low birth weight, congenital malformations, and childhood cancer for additional results from this cohort.)

Women were exposed to contaminated drinking water while pregnant and living in 75 New Jersey towns during the years 1985−1988 (Bove, 1996; Bove et al., 1995). The water contained multiple trihalomethanes, including an average of 55 ppb TCE, along with tetrachloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and benzene. A number of birth outcomes were examined for 81,532 pregnancies, which resulted in 80,938 live births and 594 fetal deaths. No association was seen for exposure to >10 ppb TCE and fetal death (OR_{adj}: 1.12). (See below for results from this study on decreased birth weight and congenital malformations.)

4.8.3.1.1.2. *Decreased birth weight, small for gestational age, and postnatal growth.*

Available data pertaining to birth weight and other growth-related outcomes come from the case-control study in Santa Clara, CA (discussed above), and by geographic-based studies as well as geographic areas with known contamination of water supplies areas in Woburn, MA; Tucson, AZ, Endicott, NY; Camp Lejeune, NC; and New Jersey.

4.8.3.1.1.2.1. *Occupational studies*. The case-control study of the relationship between solvents and adverse pregnancy outcomes discussed above (Windham et al., 1991) also examined intrauterine growth restriction (IUGR). Telephone interviews included detailed questions on occupational solvent exposure, as well as additional questions on residential solvent use. An increased risk of IUGR was observed (OR: 12.5), although this was based only on one case that was exposed to both TCE and tetrachloroethylene (also see section above on spontaneous abortion).

4.8.3.1.1.2.2. *Geographic-based studies*. The study of Woburn, MA with contaminated well water discussed above (Lagakos et al., 1986) examined birth weight. Of 3,462 live births surviving to 7 days, 220 were less than 6 pounds at birth (6.4%). No association was observed between water access and low birth weight ($p = 0.77$). (See section on spontaneous abortion for study details, and see sections on spontaneous abortion, congenital malformations, and childhood cancer for additional results from this cohort.)

An ecological analysis of well water contaminated with TCE in Tucson and birth-weight was conducted by Rodenbeck et al. (2000). The source of the exposure was a U.S. Air Force plant and the Tucson International Airport. The wells were taken out of service in 1981 after concentrations of TCE were measured in the range of <5 µg/L to 107 µg/L. The study population consisted of 1,099 babies born within census tracts between 1979 and 1981, and the comparison population consisted of 877 babies from nearby unexposed census tracts. There was a nonsignificant increased risk for maternal exposure to TCE in drinking water and very-low-birth-weight (<1,501 g) (OR: 3.3, 95% CI: 0.53−20.6). No increases were observed in the low-birth-weight (<2,501 g) (OR: 0.9) or full-term (>35-week and <46-week gestation) low-birth-weight (OR: 0.81).

The study of VOC exposure in Endicott, NY reported data on low birth weight and small for gestational age (ATSDR, 2006, see section on spontaneous abortion for study details). For births occurring during the years 1978−2002, low birth weight was slightly but statistically elevated (OR: 1.26, 95% CI: 1.00−1.59), as was small for gestational age (SGA; OR: 1.22, 95% CI: 1.02−1.45), and full-term low birth weight (OR: 1.41, 95% CI: 1.01−1.95). (Also see

sections on spontaneous abortion, congenital malformations, and childhood cancer for additional results from this cohort.)

Well water at the U.S. Marine Corps Base in Camp Lejeune, NC was identified to be contaminated with TCE, tetrachloroethylene, and 1,2-dichloroethane in April, 1982 and the wells were closed in December, 1984. ATSDR examined pregnancy outcomes among women living on the base during the years 1968−1985 (ATSDR, 1998). Compared to unexposed residents[2] (n = 5,681), babies exposed to TCE long-term[3] (n = 31) had a lower mean birth weight after adjustment for gestational age (-139 g, 90% CL = -277, -1), and babies exposed short-term[4] (n = 141) had a slightly higher mean birth weight (+70g, 90% CL = -6, 146). For the long-term group, no effect was seen for very low birth weight (<1,500 grams) or prematurity (>5 ppb, OR: 1.05). No preterm births were reported in the long-term group and those (n = 8) in the short-term group did not have an increased risk (OR: 0.7, 90% CI: 0.3−1.2). A higher prevalence of SGA[5] was seen in the long-term exposed group (n = 3; OR 1.5, 90% CL: 0.5, 3.8) compared to the short-term exposed group (OR: 1.1, 90% CI: 0.2−1.1). When the long-term group was stratified by gender, male offspring were at more risk for both reduced birth weight (−312 g, 90% CL = −632, −102) and SGA (OR: 3.9, 90% CL: 1.1−11.8). This study is limited due the mixture of chemicals in the water, as well as it small sample size. ATSDR is currently reanalyzing the findings because of an error in the exposure assessment related to the start-up date of a water treatment plant (ATSDR, 2007, 2009; GAO, 2007a, b).

Pregnancy outcomes among women were exposed to contaminated drinking water while pregnant and living in 75 New Jersey towns during the years 1985−1988 was examined by Bove et al. (Bove, 1996; Bove et al., 1995). The water contained multiple trihalomethanes, including an average of 55 ppb TCE, along with tetrachloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, 1,2-dichloroethane, and benzene. A number of birth outcomes were examined for 81,532 pregnancies, which resulted in 80,938 live births and 594 fetal deaths. A slight decrease of 17.9 grams in birth weight was seen for exposure >5 ppb, with a slight increase in risk for exposure >10 ppb (OR: 1.23), but no effect was seen for very low birth weight or SGA/prematurity (>5 ppb, OR: 1.05). However, due to the multiple contaminants in the water, it is difficult to attribute the results solely to TCE exposure. (See below for results from this study on congenital malformations.)

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[2]Unexposed residents resided at locations not classified for long-term or short-term TCE exposure.
[3]Long-term TCE exposed mothers resided at Hospital Point during 1968-1985 for at least one week prior to birth.
[4]Short-term TCE exposed mothers resided at Berkeley Manor, Midway Park, Paradise Point, and Wakins Village at the time of birth and at least 1 week during January 27 to February 7, 1985. In addition, the mother's last menstrual period occurred on or before January 31, 1985 and the birth occurred after February 2, 1985.
[5]The criteria for SGA being singleton births less than the 10th percentile of published sex-specific growth curves.

4.8.3.1.1.3. ***Congenital malformations.*** Three studies focusing on occupational solvent exposure and congenital malformations from Europe provide data pertaining to TCE. Analyses of risk of congenital malformations were also included in the studies in the four geographic areas described above (Woburn, MA; Tucson, AZ, Rocky Mountain Arsenal, CO; Endicott, NY; and New Jersey), as well as additional sites in Phoenix, AZ; and Milwaukee, WI. Specific categories of malformations examined include cardiac defects, as well as cleft lip or cleft palate.

4.8.3.1.1.3.1. *Occupational studies*. A study of 1,148 men and 969 women occupationally exposed to TCE in Finland from 1963−1976 to examined congenital malformations of offspring (Tola et al., 1980). Urinary trichloroacetic acid measurements available for 2,004 employees ranged from <10 to >500 mg/L, although 91% of the samples were below 100 mg/L. No congenital malformations were seen in the offspring of women between the ages of 15−49 years, although 3 were expected based on the national incidence. Expected number of cases for the cohort could not be estimated because the number of pregnancies was unknown.

Men from Finland occupationally exposed to organic solvents including TCE did not observe a risk of congenital malformations from paternal organic solvent exposure based on 17 cases and 35 controls exposed to TCE (OR: 0.6, 95% CI: 0.2−2.0) (Taskinen et al., 1989). (Also see section above on spontaneous abortion for study details and additional results from this cohort.)

An occupational study of 100 women who gave birth to babies born with oral cleft defects and 751 control women with normal births were examined for exposure to a number of agents including TCE during the first trimester of pregnancy (Lorente et al., 2000). All women were participants in a multicenter European case-referent study whose children were born between 1989 and 1992. Four women were exposed to TCE, resulting in two cases of cleft lip ($OR a_{dj}$: 3.21, 95% CI: 0.49−20.9), and two cases of cleft palate ($OR a_{dj}$: 4.47, 95% CI: 1.02−40.9). Using logistic regression, the increased risk of cleft palate remained high (OR: 6.7, 95% CI: 0.9−49.7), even when controlling for tobacco and alcohol consumption (OR: 7.8, 95% CI: 0.8−71.8). However, the number of cases was small, and exposure levels were not known.

4.8.3.1.1.3.2. *Geographic-based studies*. A community in Woburn, MA with contaminated well water experienced an increased incidence of adverse birth outcomes and childhood leukemia (Lagakos et al., 1986, see section on spontaneous abortion for study details). Statistically significant positive association between access to contaminated water and eye/ear birth anomalies (OR: 14.9, $p < 0.0001$), CNS/chromosomal/oral cleft anomalies (OR: 4.5, $p = 0.01$), kidney/urinary tract disorders (OR: 1.35, $p = 0.02$) and lung/respiratory tract disorders

(OR: 1.16, $p = 0.05$) were observed. There were also five cases of cardiovascular anomalies, but there was not a significant association with TCE ($p = 0.91$). However, since organogenesis occurs during gestational weeks 3−5 in humans, some of these effects could have been missed if fetal loss occurred. (Also see sections on spontaneous abortion, perinatal death, decreased birth weight, and childhood cancer for additional results from this cohort.)

A high prevalence of congenital heart disease was found within an area of Tucson Valley, AZ (Goldberg et al., 1990, see section on spontaneous abortion for study details and additional results). Of the total 707 case families included, 246 (35%) were exposed to wells providing drinking water found to be contaminated with TCE (range = 6−239 ppb), along with dichloroethylene and chromium. Before the wells were closed after the contamination was discovered in 1981, the OR of congenital heart disease was 3 times higher for those exposed to contaminated drinking water compared to those not exposed; after the wells were closed, there was no difference seen. This study observed 18 exposed cases of congenital heart disease when 16.4 would be expected (RR: 1.1). Prevalence of congenital heart disease in offspring after maternal exposure during the first trimester (6.8 in 1,000 live births) was significantly increased compared to nonexposed families (2.64 in 1,000 live births) ($p < 0.001$, 95% CI: 1.14−4.14). No difference in prevalence was seen if paternal data was included, and there was no difference in prevalence by ethnicity. In addition, no significant difference was seen for cardiac lesions.

A residential study of individuals living near the Rocky Mountain Arsenal in Colorado examined the outcomes in offspring of 75 men and 71 women exposed to TCE in drinking water (ATSDR, 2001). The risk was elevated for the nine birth defects observed (OR: 5.87, 95% CI: 0.59−58.81), including one nervous system defect, one heart defect, and one incidence of cerebral palsy. The remaining cases were classified as "other," and the authors speculate these may be based on inaccurate reports. (See above for study details and results on spontaneous abortion.)

The study of VOC exposure in Endicott, NY examined a number of birth defects during the years 1983−2000 (ATSDR, 2006, see section on spontaneous for study details). These include total reportable birth defects, structural birth defects, surveillance birth defects, total cardiac defects, major cardiac defects, cleft lip/cleft palate, neural tube defects, and choanal atresia (blocked nasal cavities). There were 56 expected cases of all birth defects and 61 were observed resulting in no elevation of risk (rate ratio, RR: 1.08, 95% CI: 0.82−1.42). There were no cases of cleft lip/cleft palate, neural tube defects, or choanal atresia. Both total cardiac defects ($n = 15$; RR: 1.94, 95% CI: 1.21−3.12) and major cardiac defects ($n = 6$; RR: 2.52, 95% CI: 1.2−5.29) were statistically increased. A follow-up study by ATSDR (2008) reported that conotruncal heart malformations were particularly elevated ($n = 4$; RR: 4.83, 95% CI: 1.81−12.89). The results remained significantly elevated (αRR: 3.74; 95% CI: 1.21−11.62)

when infants with Down syndrome were excluded from the analysis. (Also see sections on spontaneous abortion, decreased birth weight, and childhood cancer for additional results from this cohort.)

In the New Jersey study described previously, the prevalence of birth defects reported by surveillance systems was examined among the women exposed to TCE and other contaminants in water while pregnant between 1985−1988 (Bove, 1996; Bove et al., 1995). For exposure >10 ppb (n = 1,372), an increased risk, with relatively wide confidence intervals, was seen for all birth defects (OR: 2.53, 95% CI: 0.77−7.34). An increased risk was also seen for CNS defects (>10 ppb: OR: 1.68), specifically 56 cases of neural tube defects (<1−5 ppb: 1.58, 95% CI: 0.61−3.85; >10 ppb: OR: 2.53, 95% CI: 0.77−7.34). A slight increase was seen in major cardiac defects (>10 ppb: OR: 1.24, 50% CI: 0.75−1.94), including ventrical septal defects (>5 ppb: OR: 1.30, 95% CI: 0.88−1.87). An elevated risk was seen for 9 cases of oral clefts (<5 ppb: OR: 2.24, 95% CI: 1.04−4.66), although no dose-response was seen (>10 ppb, OR: 1.30). However, due to the multiple contaminants in the water, it is difficult to attribute the results solely to TCE exposure. (See above for results from this study on fetal death and decreased birth weight.)

Arizona Department of Heath Services (AZ DHS) conducted studies of contaminated drinking water and congenital malformations (<20 years old) in Maricopa County, which encompasses Phoenix and the surrounding area (AZ DHS, 1988). TCE contamination was associated with elevated levels of deaths in children less than 20 years old due to total congenital anomalies in East Central Phoenix from 1966−1969 (RR: 1.4, 95% CI: 1.1−1.7), from 1970−1981 (RR: 1.5, 95% CI: 1.3−1.7), and from 1982−1986 (RR: 2.0, 95% CI: 1.5−2.5), as well as in other areas of the county. (See below for results from this study on childhood leukemia.)

A study was conducted of children born 1997−1999 with congenital heart defects in Milwaukee, WI (Yauck et al., 2004). TCE emissions data were ascertained from state and U.S. EPA databases, and distance between maternal residence and the emission source was determined using a GIS. Exposure was defined as those within 1.32 miles from at least one site. Results showed that an increased risk of congenital heart defects was seen for the offspring of exposed mothers 38 years old or older (OR: 6.2, 95% CI: 2.6−14.5), although an increased risk was also seen for offspring of unexposed mothers 38 years old or older (OR: 1.9, 95% CI: 1.1−3.5), and no risk was seen for offspring of exposed mothers younger than 38 years (OR: 0.9, 95% CI: 0.6−1.2). The authors speculate that studies that did not find a risk only examined younger mothers. The authors also note that statistically-significant increased risk was seen for mothers with preexisting diabetes, chronic hypertension, or alcohol use during pregnancy.

An abstract reported that twenty-eight people living in a Michigan town were exposed for 5−10 years to 8−14 ppm TCE in well water (Bernad et al., 1987, abstract). One child was born with multiple birth defects, with no further details.

4.8.3.1.1.4. ***Other adverse birth outcomes.*** TCE was previously used as a general anesthetic during pregnancy. One study measured the levels of TCE in maternal and newborn blood after use during 34 vaginal childbirths (Beppu, 1968). TCE was administered through a vaporizer from two to 98 minutes (mean 34.7 minutes) at volumes from 2 to 8 mL (mean 4.3 mL). Mean blood TCE concentrations were 2.80 ± 1.14 mg/dL in maternal femoral arteries; 2.36 ± 1.17 mg/dL in maternal cubital veins; 1.83 ± 1.08 mg/dL in umbilical vein; and 1.91 ± 0.95 mg/dL in the umbilical arteries. A significant correlation was seen for maternal arterial blood and infants' venous blood, and the concentration of the fetal blood was lower than that of the mother. Of these newborns, one had asphyxia and three "sleepy babies" had Apgar scores of 5 to 9; however, these results could not be correlated to length of inhalation and there was no difference in the TCE levels in the mother or newborn blood compared to those without adverse effects. Discussion included delayed newborn reflexes (raising the head and buttocks, bending the spine, and sound reflex), blood pressure, jaundice, and body weight gain; however, the results were compared to newborns exposed to other compounds, not to an unexposed population. This study also examined the concentration of TCE in one mother at 22-weeks gestation exposed for four minutes, after which the fetus was "artificially delivered." Maternal blood concentration was 3.0 mg/dL, and 0.9 mg/dL of TCE was found in the fetal heart, but not in other organs.

Another study of TCE administered during childbirth to the mother as an analgesic examined perinatal measures, including fetal pH, fetal partial pressure carbon dioxide (PCO_2) fetal base deficit, fetal partial pressure oxygen (PO_2), Apgar scores, and neonatal capillary blood (Phillips and Macdonald, 1971). The study consisted of 152 women whose fetus was considered to be at risk for hypoxia during labor. Out of this group, 51 received TCE (amount and route of exposure not reported). TCE caused fetal pH to fall more, base deficit increased more, and PO_2 fell more than the control group by 4-fold or more compared to other analgesics used.

4.8.3.1.2. ***Postnatal developmental outcomes.***

4.8.3.1.2.1. ***Developmental neurotoxicity.*** The studies examining neurotoxic effects from TCE exposure are discussed in Section 4.3, and the human developmental neurotoxic effects are reiterated here.

4.8.3.1.2.1.1. *Occupational studies*. An occupational study examined the neurodevelopment of the offspring of 32 women exposed to various organic solvents during pregnancy (Laslo-Baker et

al., 2004; Till et al., 2001). Three of these women were exposed to TCE; however, no levels were measured and the results for examined outcomes are for total organic solvent exposure, and are not specific to TCE.

4.8.3.1.2.1.2. *Geographic-based studies*. A study of three residential cohorts (Woburn, MA, Alpha, OH, and Twin Cities, MN) examined the neurological effects of TCE exposure in drinking water (White et al., 1997). For Woburn, MA, 28 individuals ranging from 9−55 years old were assessed, with exposure from a tanning factor and chemical plant at levels 63−400 ppb for <1 to 12 years; the time between exposure and neurological examination was about 5 years. In this cohort, six of thirteen children (46%) had impairments in the verbal naming/language domain. For Alpha, OH, 12 individuals ranging from 12−68 years old were assessed, with exposure from degreasing used at a manufacturing operation at levels 3.3−330 ppb for 5−17 years; the time between exposure and neurological examination was 5−17 years. In this cohort, one of two children (50%) had impairments in the verbal naming/language domain. For Twin Cities, MN, 14 individuals ranging from 8−62 years old were assessed, with exposure from an army ammunition plant at levels 261−2,440 ppb for 0.25−25 years; the time between exposure and neurological examination was 4−22 years. In this cohort, four of four children (100%) had impairments in the verbal naming/language, memory, and academic domains and were diagnosed with moderate encephalopathy; and three of four children (75%) performed poorly on the WRAT-R Reading and Spelling and WAIS-R Information tests.

A case-control study was conducted to examine the relationship between multiple environmental agents and autism spectrum disorder (ASD) (Windham et al., 2006). Cases (n = 284) and controls (n = 657) were born in 1994 in the San Francisco Bay Area. Cases were diagnosed before age nine. Exposure was determined by geocoding births to census tracts, and linking to hazardous air pollutants (HAPs) data. An elevated risk was seen for TCE in the upper 3rd quartile (OR: 1.37, 95% CI: 0.96−1.95), and a statistically significant elevated risk was seen for the upper 4th quartile (OR: 1.47, 95% CI: 1.03−2.08).

The Trichloroethylene Subregistry (Burg et al., 1995; Burg and Gist, 1999), including 948 children <18 years old from 13 sites located in 3 states, was examined for any association of ingestion of drinking water contaminated with TCE and various health effects (Burg et al., 1995; Burg and Gist, 1999; ATSDR, 2003a). Exposure groups included (1) maximum TCE exposure, (2) cumulative TCE exposure, (3) cumulative chemical exposure, and (4) duration of exposure. Exposed children 0−9 years old had statistically increased hearing impairment compared to controls (RR: 2.13, 99% CI: 1.12−4.07), with children <5 having a 5.2-fold increase over controls. Exposed children 0−9 years old also had statistically increased speech impairment (RR: 2.45, 99% CI: 1.31−4.58). In addition, anemia and other blood disorders were statistically

higher for males 0−9 years old. The authors noted that exposure could have occurred prenatally or postnatally. There was further analysis on the 116 exposed children and 182 controls who were under 10 years old at the time that the baseline study was conducted by ATSDR. This analysis did not find a continued association with speech and hearing impairment in these children; however, the absence of acoustic reflexes (contraction of the middle ear muscles in response to sound) remained significant (ATSDR, 2003a). No differences were seen when stratified by prenatal and postnatal exposure.

Twenty-eight people living in a Michigan town were exposed for 5−10 years to 8−14 ppm TCE in well water (Bernad et al., 1987). Ten adults and 12 children completed a questionnaire on neurotoxic endpoints. Nine of the 12 children had poor learning ability, aggressive behavior, and low attention span.

4.8.3.1.2.2. ***Developmental immunotoxicity.*** The studies examining human immunotoxic effects from TCE exposure are discussed in Section 4.6.1. The studies reporting developmental effects are reiterated briefly here.

Two studies focused on immunological development in children after maternal exposure to VOCs (Lehmann et al., 2001, 2002). The first examined premature neonates (1,500−2,500 g) and neonates at risk of atopy (cord blood IgE >0.9 kU/L; double positive family atopy history) at 36 months of age (Lehmann et al., 2001). Median air level in child's bedroom measured 0.42 $\mu g/m^3$. There was no association with allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells. The second examined healthy, full-term neonates (≥2,500 g; ≥37 weeks gestation) born in Lepzig, Germany (Lehmann et al., 2002). Median air level in the child's bedroom 3−4 weeks after birth measured 0.6 $\mu g/m^3$. A significant reduction of Th1 IL-2 producing T-cells was observed.

Byers et al. (1988) observed altered immune response in family members of children diagnosed with leukemia in Woburn, MA (Lagakos et al., 1986, see below for results of this study). The family members included 13 siblings under 19 years old at the time of exposure; however, an analysis looking at only these children was not done. This study is discussed in further detail in Section 4.6.1.

4.8.3.1.2.3. ***Other developmental outcomes.*** A study demonstrated the adverse effects of TCE used as an anesthetic in children during operations during 1964 in Poland to repair developmental defects of the jaw and face (Jasinka, 1965, translation). Fifty-five children ranging from 6 months to 10 years old were anesthetized with at least 10 mL TCE placed into an evaporator. Bradycardia occurred in 2 children, an accelerated heart rate of 20−25 beats per minute occurred in 7 children, no arrhythmia was observed, and arterial blood pressure remained

steady or dropped by 10 mmHG only. Respiratory acceleration was observed in 25 of the children, and was seen more in infants and younger children.

4.8.3.1.2.4. ***Childhood cancer.*** Several studies of parental occupational exposure were conducted in North America and the United Kingdom to determine an association with childhood cancer. A number of geographic-based studies were conducted in California; New Jersey; Woburn, MA; Endicott, NY; Phoenix, AZ; and Tucson, AZ. Specific categories of childhood cancers examined include leukemia, non-Hodgkin's lymphoma, and CNS tumors.

4.8.3.1.2.4.1. *Occupational studies*. Brain tumors in 98 children less than 10 years old at diagnosis from 1972−1977 in Los Angeles County have been observed in the offspring of fathers (Peters et al., 1981, 1985). Exposure was determined by questionnaire. Two cases with TCE exposure were reported: one case of oligodendroglioma in an 8-year-old whose father was a machinist, and astrocytoma in a 7-year-old whose father was an inspector for production scheduling and parts also exposed to methyl ethyl ketone (Peters et al., 1981). Peters et al. (1985) also briefly mentioned 5 cases and no controls of paternal exposure to TCE and brain tumors in the offspring (resulting in an inability to calculate an odds ratio), but without providing any additional data.

A case-control study was conducted to assess an association between parental occupational exposure and neuroblastoma diagnosed in offspring <19 years old in the United States and Canada from May 1992 to April 1994 (De Roos et al., 2001). Paternal self-reported exposure to TCE was reported in 22 cases and 12 controls, resulting in an elevated risk of neuroblastoma in the offspring (OR: 1.4, 95%CI: 0.7−2.9). Maternal exposure to TCE was not reported.

A case-control study of parental occupational exposure and childhood leukemia was conducted in Los Angeles County (Lowengart et al., 1987). Children (61 boys and 62 girls) diagnosed less than 10 years old (mean age 4 years) from 1980 to 1984 were included in the analysis. Paternal occupation exposure to TCE was elevated for one year preconception (OR: 2.0, $p = 0.16$), prenatal (OR: 2.0, $p = 0.16$), and postnatal (OR: 2.7, $p = 0.7$). Maternal exposure to TCE was not reported.

A case-control study children diagnosed with acute lymphoblastic leukemia (ALL) examined parental occupational exposure to hydrocarbons in the United States and Canada (Shu et al., 1999). Children were under the age of 15 years at diagnosis during the years 1989 to 1993. Cases were confirmed with a bone marrow sample. 1,842 case-control pairs were given questionnaires on maternal and paternal exposures, resulting in 15 cases and 9 controls maternally exposed and 136 cases and 104 controls paternally exposed to TCE. There was an

increased but nonsignificant risk for maternal exposure to TCE during preconception (OR: 1.8, 95% CI: 0.6−5.2), pregnancy (OR: 1.8, 95% CI: 0.5−6.4), postnatally (OR: 1.4, 95% CI: 0.5−4.1), or any of these periods (OR: 1.8, 95% CI: 0.8−4.1). However, there was no increased risk for paternal exposure to TCE.

Occupational exposure in communities in the United Kingdom was examined to determine an association with leukemia and non-Hodgkin's lymphoma diagnosed in the offspring (McKinney et al., 1991). Paternal occupational exposure was elevated for exposure occurring during preconception (OR: 2.27, 95% CI: 0.84−6.16), prenatal (OR: 4.40, 95% CI: 1.15−21.01), and postnatal (OR: 2.66, 95% CI: 0.82−9.19). Risk from maternal preconception exposure was not elevated (OR: 1.16, 95% CI: 0.13−7.91). However, the number of cases examined in this study was low, particularly for maternal exposure.

4.8.3.1.2.4.2. *Geographic-based studies*. A California community exposed to TCE (0.09−97 ppb) in drinking water from contaminated wells was examined for cancer (Morgan and Cassady, 2002). A specific emphasis was placed on the examination of 22 cases of childhood cancer diagnosed before 15 years old. However, the incidence did not exceed those expected for the community for total cancer (SIR: 0.83, 99% CI: 0.44−1.40), CNS cancer (SIR: 1.05, 99% CI: 0.24−2.70), and leukemia (SIR: 1.09, 99% CI: 0.38−2.31).

An examination of drinking water was conducted in four New Jersey counties to determine an association with leukemia and non-Hodgkin's lymphoma (Cohn et al., 1994). A number of contaminants were reported, including VOCs and trihalomethanes. TCE was found as high as 67 ppb, and exposure categories were assigned to be >0.1, 0.1−5 and >5 ppb. A significantly elevated dose-response risk for ALL was observed for girls diagnosed before 20 years old (RR: 3.36, 95% CI: 1.29−8.28), which was increased among girls diagnosed before 5 years old (RR:4.54, 95% CI: 1.47−10.6). A significantly elevated dose-response risk for girls was also observed for total leukemia (RR: 1.43, 95% CI: 1.07−1.98).

The Woburn, MA community with contaminated well water experienced an increase in the incidence of childhood leukemia (Costas et al., 2002; Cutler et al., 1986; Lagakos et al., 1986; MA DPH, 1997). An initial study examined twelve cases of childhood leukemia diagnosed in children less than 15 years old between 1969−1979, when 5.2 cases were expected, and a higher risk was observed in boys compared to girls; however, no factors were observed to account for this increase (Cutler et al., 1986). Another study observed statistically significant positive association between access to contaminated water and 20 cases of childhood cancer were observed for both cumulative exposure metric (OR: 1.39, $p = 0.03$), and none versus some exposure metric (OR: 3.03, $p = 0.02$) (Lagakos et al., 1986). Massachusetts Department of Public Health (MA DPH, 1997) conducted a case-control study of children less than 20 years old

living in Woburn and diagnosed with leukemia between 1969 and 1989 (n = 21) and observed that consumption of drinking water increased the risk of leukemia (OR: 3.03, 95% CI: 0.82−11.28), with the highest risk from exposure during fetal development (OR: 8.33, 95% CI: 0.73−94.67). This study found that paternal occupational exposure to TCE was not related to leukemia in the offspring (MA DPH, 1997). In the most recent update, Costas et al. (2002) reported that between the years 1969 and 1997, 24 cases of childhood leukemia were observed when 11 were expected. Risk was calculated for cumulative exposure to contaminated drinking water two years prior to conception (OR_{adj}: 2.61, 95% CI: 0.47−14.97), during pregnancy (OR_{adj}: 8.33, 95% CI: 0.73−94.67), postnatal (OR_{adj}: 1.18, 95% CI: 0.28−5.05), and any of these time periods (OR_{adj}: 2.39, 95% CI: 0.54−10.59). A dose response was observed during pregnancy only. Cases were more likely to be male (76%), <9 years old at diagnosis (62%), breast-fed (OR: 10.17, 95% CI: 1.22−84.50), and exposed during pregnancy (adjusted OR: 8.33, 95% CI: 0.73−94.67). A dose-response was seen during the pregnancy exposure period, with the most exposed having an adjusted OR of 14.30 (95% CI: 0.92−224.52). Other elevated risks observed included maternal alcohol intake during pregnancy (OR: 1.50, 95% CI: 0.54−4.20), having a paternal grandfather diagnosed with cancer (OR: 2.01, 95% CI: 0.73−5.58), father employed in a high risk industry (OR: 2.55, 95% CI: 0.78−8.30), and public water being the subject's primary beverage (OR: 3.03, 95% CI: 0.82−11.28). (Also see sections on spontaneous abortion, perinatal death, decreased birth weight, and congenital malformations for additional results from this cohort.)

The study of VOC exposure in Endicott, NY discussed above observed fewer than six cases of cancer that were diagnosed between 1980 and 2001 in children less than 20 years old, and did not exceed expected cases or types (ATSDR, 2006). (See section on spontaneous abortion for study details, and sections on spontaneous abortion, decreased birth weight, and congenital malformations for additional results from this cohort.)

The AZ DHS conducted a number of studies of contaminated drinking water and 189 cases of childhood cancer (<20 years old) (AZ DHS, 1988, 1990a, b, c, 1997). In Maricopa County, which encompasses Phoenix and the surrounding area, TCE contamination (8.9 and 29 ppb in two wells) was associated with elevated levels of childhood leukemia (n = 67) in west central Phoenix during 1965−1986 (SIR: 1.67, 95% CI: 1.20−2.27) and 1982−1986 (SIR: 1.91, 95% CI: 1.11−3.12), but did not observe a significant increase in total childhood cancers, lymphoma, brain/CNS, or other cancers during these time periods (AZ DHS, 1990a). (See above for results from this study on congenital anomalies.) A follow-up study retrospectively asked parents about exposures and found that residence within 2 miles of wells contaminated with TCE was not a risk factor for childhood leukemia, but identified a number of other risk factors (AZ DHS, 1997). A further study of East Phoenix, reported on TCE contamination found along

with 1,1,1-trichloroethane and 25 other contaminants in well water (levels not reported) and found no increase in incidence of childhood leukemia (SIR: 0.85, 95% CI: 0.50−1.35) based on 16 cases (AZ DHS, 1990b). There were also 16 cases of other types of childhood cancer, but were too few to be analyzed separately. In Pima County, which encompasses Tucson and the surrounding area, TCE was found in drinking wells (1.1−239 ppb), along with 1,1-dichloroethylene (DCE), chloroform and chromium and found a nonstatistically elevated risk of leukemia was observed (SIR: 1.50, 95% CI: 0.76−2.70), but no risk was observed for testicular cancer, lymphoma, or CNS/brain cancer (AZ DHS, 1990c).

4.8.3.1.3. ***Summary of human developmental toxicity.*** Epidemiological developmental studies examined the association between TCE exposure and a number of prenatal and postnatal developmental outcomes. Prenatal developmental outcomes examined include spontaneous abortion and perinatal death; decreased birth weight, small for gestational age, and postnatal growth; congenital malformations; and other adverse birth outcomes. Postnatal developmental outcomes examined include developmental neurotoxicity, developmental immunotoxicity, other developmental outcomes, and childhood cancer related to TCE exposure.

More information on developmental outcomes is expected. A follow-up study of the Camp Lejeune cohort (ATSDR, 1998) for birth defects and childhood cancers was initiated in 1999 (ATSDR, 2003b) and expected to be completed soon (GAO, 2007a, b; ATSDR, 2009). Out of a total of 106 potential cases of either birth defects or childhood cancer, 57 have been confirmed and will constitute the cases. These will be compared 548 control offspring of mothers who also lived at Camp Lejeune during their pregnancy from 1968−1985. As part of this study, a drinking water model was developed to determine a more accurate level and duration of exposure to these pregnant women (ATSDR, 2007). Additional health studies have been suggested, including adverse neurological or behavioral effects or pregnancy loss.

4.8.3.2. ***Animal Developmental Toxicology Studies***

A number of animal studies have been conducted to assess the potential for developmental toxicity of TCE. These include studies conducted in rodents by prenatal inhalation or oral exposures (summarized in Tables 4-85 and 4-86), as well as assessments in nonmammalian species (e.g., avian, amphibian, and invertebrate species) exposed to TCE during development. Studies have been conducted that provide information on the potential for effects on specific organ systems, including the developing nervous, immune, and pulmonary systems. Additionally, a number of research efforts have focused on further characterization of the mode of action for cardiac malformations that have been reported to be associated with TCE exposure.

Table 4-85. Summary of mammalian *in vivo* developmental toxicity studies—inhalation exposures

Reference	Species/strain/ sex/number	Exposure level/ duration	NOAEL; LOAEL [a]	Effects
Carney et al., 2006	Rat, Sprague-Dawley, females, 27 dams/group	0, 50, 150, or 600 ppm (600 ppm = 3.2 mg/L)[b] 6 h/d; GD 6−20	Mat. NOAEL: 150 ppm Mat. LOAEL: 600 ppm	↓ BW gain (22% less than control) on GD 6−9 at 600 ppm.
			Dev. NOAEL: 600 ppm	No evidence of developmental toxicity, including heart defects.
Dorfmueller et al., 1979	Rat, Long-Evans, females, 30 dams/group	0 or 1,800 ± 200 ppm (9,674 ± 1,075 mg/m^3)[b] 2 wks, 6 h/d, 5 d/wk; prior to mating and/or on GD 0−20	Mat. NOAEL: 1,800 ± 200 ppm	No maternal abnormalities.
			Dev. LOAEL: 1,800 ± 200 ppm	Sig. ↑ skeletal and soft tissue anomalies in fetuses from dams exposed during pregnancy only. No sig. treatment effects on behavior of offspring 10, 20, or 100 d postpartum. BW gains sig. ↓ in pups from dams with pregestational exposure.
Hardin et al., 1981	Rat, Sprague-Dawley, female, nominal 30/group	0 or 500 ppm 6−7 h/d; GD 1−19	Mat. NOAEL: 500 ppm	No maternal toxicity
			Dev. NOAEL: 500 ppm	No embryonic or fetal toxicity.
	Rabbit, New Zealand white, female, nominal 20/group	0 or 500 ppm 6−7 h/d; GD 1−24	Mat. NOAEL: 500 ppm	No maternal toxicity.
			Dev. LOAEL: 500 ppm	Hydrocephaly observed in 2 fetuses of 2 litters, considered equivocal evidence of teratogenic potential.
Healy et al., 1982	Rat, Wistar, females, 31−32 dams/group	0 or 100 ppm 4 h/d; GD 8−21	Mat. NOAEL: 100 ppm	No maternal abnormalities.
			Dev. LOAEL: 100 ppm	Litters with total resorptions sig. ↑. Sig. ↓ fetal weight, and ↑ bipartite or absent skeletal ossification centers.
Schwetz et al., 1975	Rat, Sprague-Dawley, female, 20−35/group Mouse, Swiss-Webster, females, 30−40 dams/group	0 or 300 ppm 7 h/d; GD 6−15	Mat. LOAEL: 300 ppm	4−5% ↓ maternal BW
			Dev. NOAEL: 300 ppm	No embryonic or fetal toxicity; not teratogenic.
Westergren et al., 1984	Mouse, NMRI, male and female, 6−12 offspring/group	0 or 150 ppm 24 h/d; 30 d (during 7 d of mating and until GD 22)	Dev. LOAEL: 150 ppm[c]	Specific gravity of brains sig. ↓ at PND 0, 10, and 20−22. Similar effects at PND 20−22 in occipital cortex and cerebellum. No effects at 1 month of age.

[a]NOAEL and LOAEL are based upon reported study findings. Mat. = maternal; Dev. = developmental.
[b]Dose conversions provided by study author(s).
[c]Parental observations not reported.

Table 4-86. Ocular defects observed (Narotsky et al., 1995)

Dose TCE (mg/kg/d)	Incidence (no. affected pups/total no. pups)*	Percent pups with eye defects
0	1/197	0.51
10.1	0/71	0.00
32	0/85	0.00
101	3/68	4.41
320	3/82	3.66
475	6/100	6.00
633	6/100	6.00
844	7/58	12.07
1,125	12/44	27.27

*Reported in Barton and Das (1996).

4.8.3.2.1. *Mammalian studies*

Studies that have examined the effects of TCE on mammalian development following either inhalation or oral exposures are described below and summarized in Tables 4-85 and 4-87, respectively.

Table 4-87. Summary of mammalian *in vivo* developmental toxicity studies—oral exposures

1

Reference	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Blossom and Doss, 2007	Mouse, MRL +/+, dams and both sexes offspring, 3 litters/group, 8−12 offspring/group	0, 0.5, or 2.5 mg/mL Parental mice and/or offspring exposed from GD 0 to 7−8 months of age	Drinking water	Dev. LOAEL = 0.5 mg/mL[b]	At 0.5 mg/mL: Sig ↓ postweaning weight; sig.↑ IFNγ produced by splenic CD4+ cells at 5−6 wks; sig ↓ splenic CD8+and B220+ lymphocytes; sig.↑ IgG2a and histone; sig. altered CD4-/CD8- and CD4+/CD8+ thymocyte profile. At 2.5 mg/mL: Sig ↓ postweaning weight; sig.↑ IFNγ produced by splenic CD4+ and CD8+ cells at 4−5 and 5−6 wks; sig ↓ splenic CD4+, CD8+, and B220+ lymphocytes; sig. altered CD4+/CD8+ thymocyte profile.
Blossom et al., 2008	Mouse, MRL +/+, dams and both sexes offspring, 8 litters/group, 3−8 offspring/group	0 or 0.1 mg/mL (maternal dose = 25.7 mg/kg/d; offspring PND 24−42 dose—31.0 mg/kg/d Parental mice and/or offspring exposed from GD 0 to PND 42	Drinking water	Dev. LOAEL = 1,400 ppb[b]	At 0.1 mg/mL: at PND 20, sig. ↑ thymocyte cellularity and distribution, associated with sig. ↑ in thymocyte subset distribution; sig. ↑ reactive oxygen species generation in total thymocytes; sig. ↑ in splenic CD4+ T-cell production of IFN-γ and IL-2 in females and TNF-α in males at PND 42. Significantly impaired nest-building behaviors at PND 35. Increased aggressive activities, and increased oxidative stress and impaired thiol status in the cerebellar tissue of male offspring at PND 40.

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Table 4-87. Summary of mammalian *in vivo* developmental toxicity studies—oral exposures (continued)

Reference	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Collier et al., 2003	Rat, Sprague-Dawley, female, no. dams/group not reported	0, 0.11, or 1.1 mg/mL (0, 830, or 8,300 μgM)[c] GD 0−11	Drinking water	Dev. LOEL: 0.11 mg/mL	Embryos collected between GD 10.5 and 11. Gene expression at 1.1 mg/mL TCE: 8 housekeeping genes ↑, and one gene ↓; 3 stress response genes ↑, IL-10 ↓; 2 cyto-skeletal/cell adhesion/blood related genes ↑, 3 genes ↓; 2 heart-specific genes ↑. Effects at 0.11 mg/mL reduced considerably. Two possible markers for fetal TCE exposure identified as Serca-2 Ca^{+2} ATPase and GPI-p137.
Cosby and Dukelow, 1992	Mouse, B6D2F1, female, 28−62 dams/group	0, 24, or 240 mg/kg/d GD 1−5, 6−10, or 11−15	Gavage in corn oil	Mat. NOAEL: 240 mg/kg/d	No maternal toxicity.
				Dev. NOAEL: 240 mg/kg/d	No effects on embryonic or fetal development.
Dawson, et al., 1993	Rat, Sprague-Dawley, 116 females allocated to 11 groups	0, 1.5, or 1,100 ppm 2 mo before mating and/or during gestation	Drinking water	Mat. NOAEL: 1,100 ppm	No maternal toxicity.
				Dev. LOAEL: 1.5 ppm	Sig. ↑ in heart defects, primarily atrial septal defects, found at both dose levels in groups exposed prior to pregnancy and during pregnancy, as well as in group exposed to 1,100 ppm dose during pregnancy only. No sig. ↑ in congenital heart defects in groups exposed prior to pregnancy only.
Fisher et al., 2001; Warren et al., 2006	Rat, Sprague-Dawley, female, 20−25 dams/group	0 or 500 mg/kg/d GD 6−15	Gavage in soybean oil	Mat. NOAEL: 500 mg/kg/d	No maternal toxicity.
				Dev. NOAEL: 500 mg/kg/d	No developmental toxicity. The incidence of heart malformations for fetuses from TCE-treated dams (3−5%) did not differ from neg. controls. No eye defects observed.

Table 4-87. Summary of mammalian *in vivo* developmental toxicity studies—oral exposures (continued)

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% micro-encapsulated 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 devise.
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)[c] Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOAEL: 312 mg/L[b]	Sig. ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.
Johnson et al., 2003	Rat, Sprague-Dawley, female, 9−13/group, 55 in control group	0, 2.5 ppb, 250 ppb, 1.5 ppm, or 1,100 ppm (0, 0.00045, 0.048, 0.218, or 129 mg/kg/d)[c] GD 0−22	Drinking water	Dev. NOAEL: 2.5 ppb Dev. LOAEL: 250 ppb[b]	Sig. ↑ in percentage of abnormal hearts and the percentage of litters with abnormal hearts at ≥250 ppb.
Narotsky et al., 1995	Rat, Fischer 344, females, 8−12 dams/group	0, 10.1, 32, 101, 320, 475, 633, 844, or 1,125 mg/kg/d GD 6−15	Gavage in corn oil	Mat. LOAEL: 475 mg/kg/d	Sig. dose-related ↓ dam BW gain at all dose levels on GD 6−8 and 6−20. Delayed parturition at ≥475 mg/kg/d; ataxia at ≥633 mg/kg/d; mortality at 1,125 mg/kg/d.

Table 4-87. Summary of mammalian *in vivo* developmental toxicity studies—oral exposures (continued)

Reference	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Narotsky et al., 1995 (continued)				Dev. NOAEL: 32 mg/kg/d Dev. LOAEL: 101 mg/kg/d	↑ full litter resorption and postnatal mortality at ≥425 mg/kg/d. Sig. prenatal loss at 1,125 mg/kg/d. Pup BW ↓ (not sig.) on PND 1 and 6. Sig. ↑ in pups with eye defects at 1,125 mg/kg/d. Dose-related (not sig.) ↑ in pups with eye defects at ≥101 mg/kg/d.
Narotsky and Kavlock, 1995	Rat, Fischer 344, females, 16−21 dams/group	0, 1,125, or 1,500 mg/kg/d GD 6−19	Gavage in corn oil	Mat. LOAEL: 1,125 mg/kg/d	Ataxia, ↓ activity, piloerection; dose-related ↓ BW gain.
				Dev. LOAEL: 1,125 mg/kg/d	Sig. ↑ full litter resorptions, ↓ live pups/litter; sig. ↓ pup BW on PND 1; sig. ↑ incidences of microophthalmia and anophthalmia.
Noland-Gerbec et al., 1986	Rat, Sprague-Dawley, females, 9−11 dams/group	0 or 312 mg/L (Avg. total intake of dams: 825 mg TCE over 61 d.)[c] Dams (and pups) exposed from 14 d prior to mating until end of lactation.	Drinking water	Dev. LOEL: 312 mg/L[b]	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.
Peden-Adams et al., 2006	Mouse, B6C3F1, dams and both sexes offspring, 5 dams/group; 5−7 pups/group at 3 wks; 4−5 pups/sex/group at 8 wks	0, 1,400, or 14,000 ppb Parental mice and/or offspring exposed during mating, and from GD 0 thru 3 or 8 wks of age	Drinking water	Dev. LOAEL: 1,400 ppb[b]	At 1,400 ppb: Suppressed plaque-forming cell (PFC) responses in males at 3 and 8 wks of age and in females at 8 wks of age. Delayed hypersensitivity response increased at 8 wks of age in females. At 14,000 ppb: Suppressed PFC responses in males and females at 3 and 8 wks of age. Splenic cell population decreased in 3 wk old pups. Increased thymic T-cells at 8 wks of age. Delayed hypersensitivity response increased at 8 wks of age in males and females.

Table 4-87. Summary of mammalian *in vivo* developmental toxicity studies—oral exposures (continued)

Reference	Species/strain/ sex/number	Dose level/ exposure duration	Route/ vehicle	NOAEL; LOAEL[a]	Effects
Peden-Adams et al., 2008	Mouse, MRL +/+, dams and both sexes offspring, unknown no. litters/group, 6−10 offspring/sex/group	0, 1,400, or 14,000 ppb (vehicle = 1% emulphore) Parental mice and/or offspring exposed from GD 0 to 12 months of age	Drinking water	Dev. LOAEL = 1,400 ppb[b]	At 1,400 ppb: splenic CD4-/CD8- cells sig.↑ in females; thymic CD4+/CD8+ cells sig. ↓ in males; 18% ↑ in male kidney weight. At 14,000 ppb: thymic T-cell subpopulations (CD8+, CD4/CD8-, CD4+) sig. ↓ in males.
Taylor et al., 1985	Rat, Sprague-Dawley, females, no. dams/group not reported	0, 312, 625, or 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[b]	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. Mat. = Maternal; Dev. = Developmental.
[b]Dose conversions provided by study author(s).
[c]Maternal observations not reported.

4.8.3.2.1.1. *Inhalation exposures*. Dorfmueller et al. (1979) conducted a study in which TCE was administered by inhalation exposure to groups of approximately 30 female Long-Evans hooded rats at a concentration of 1,800 ± 200 ppm before mating only, during gestation only, or throughout the premating and gestation periods. Half of the dams were killed at the end of gestation and half were allowed to deliver. There were no effects on body weight change or relative liver weight in the dams. The number of corpora lutea, implantation sites, live fetuses, fetal body weight, resorptions, and sex ratio were not affected by treatment. In the group exposed only during gestation, a significant increase in four specific sternebral, vertebral, and rib findings, and a significant increase in displaced right ovary were observed upon fetal skeletal and soft tissue evaluation. Mixed function oxidase enzymes (ethoxycoumarin and ethoxyresorbin) which are indicative of cytochrome P450 and P448 activities, respectively, were measured in the livers of dams and fetuses, but no treatment-related findings were identified. Postnatal growth was significantly ($p < 0.05$) decreased in the group with gestation-only exposures. Postnatal behavioral studies, consisting of an automated assessment of ambulatory response in a novel

environment on postnatal days 10, 20, and 100, did not identify any effect on general motor activity of offspring following in utero exposure to TCE.

In a study by Schwetz et al. (1975), pregnant Sprague-Dawley rats and Swiss Webster mice (30−40 dams/group) were exposed to TCE via inhalation at a concentration of 300 ppm for 7 hours/day on gestation days 6−15. The only adverse finding reported was a statistically significant 4−5% decrease in maternal rat body weight. There were no treatment related effects on pre- and postimplantation loss, litter size, fetal body weight, crown-rump length, or external, soft tissue, or skeletal findings.

Hardin et al. (1981) summarized the results of inhalation developmental toxicology studies conducted in pregnant Sprague-Dawley rats and New Zealand white rabbits for a number of industrial chemicals, including TCE. Exposure concentrations of 0 or 500 ppm TCE were administered for 6−7 hours/day, on gestations days 1−19 (rats) or 1−24 (rabbits), and cesarean sections were conducted on gestation days 21 or 30, respectively. There were no adverse findings in maternal animals. No statistically significant increase in the incidence of malformations was reported for either species; however, the presence of hydrocephaly in two fetuses of two TCE-treated rabbit litters was interpreted as a possible indicator of teratogenic potential.

Healy et al. (1982) did not identify any treatment-related fetal malformations following inhalation exposure of pregnant inbred Wistar rats to 0 or 100 ppm (535 mg/m^3) on GD 8−21. In this study, significant differences between control and treated litters were observed as an increased incidence of total litter loss ($p < 0.05$), decreased mean fetal weight ($p < 0.05$), and increased incidence of minor ossification variations ($p = 0.003$) (absent or bipartite centers of ossification).

Carney et al. (2006) investigated the effects of whole-body inhalation exposures to pregnant Sprague-Dawley rats at nominal (and actual) chamber concentrations of 0, 50, 150, or 600 ppm TCE for 6 hours/day, 7 days/week on gestation days 6−20. This study was conducted under Good Laboratory Practice regulations according to current U.S. EPA and Organisation for Economic Co-operation and Development (OECD) regulatory testing guidelines (i.e., OPPTS 870.3700 and OECD GD 414). Maternal toxicity consisted of a statistically significant decrease (22%) in body weight gain during the first 3 days of exposure to 600-ppm TCE, establishing a no-observed-effect concentration (NOEC) of 150 ppm for dams. No significant difference between control and TCE-treated groups was noted for pregnancy rates, number of corpora lutea, implantations, viable fetuses per litter, percent pre- and postimplantation loss, resorption rates, fetal sex ratios, or gravid uterine weights. External, soft tissue, and skeletal evaluation of fetal specimens did not identify any treatment-related effects. No cardiac malformations were identified in treated fetuses. The fetal NOEC for this study was established at 600 ppm.

Westergren et al. (1984) examined brain specific gravity of NMRI mice pups following developmental exposures to TCE. Male and female mice were separately exposed 24 hours/day (except for limited periods of animal husbandry activities) to 0- or 150-ppm TCE for 30 days and mated during exposure for 7 days. Exposure of the females was continued throughout gestation, until the first litter was born. Offspring (6−12/group; litter origin not provided in report) were terminated by decapitation on PND 1, 10, 21−22, or 30. The specific gravity of the brain frontal cortex, cortex, occipital cortex, and cerebellum were measured. The cortex specific gravity was significantly decreased at PND 1 ($p < 0.001$) and 10 ($p < 0.01$) in pups from exposed mice. There were also significant differences ($p < 0.05$) in the occipital cortex and cerebellum at PND 20−22. This was considered suggestive of delayed maturation. No significant differences between control and treated pups were observed at one month of age.

4.8.3.2.1.2. ***Oral exposures*****.** A screening study conducted by Narotsky and Kavlock (1995) assessed the developmental toxicity potential of a number of pesticides and solvents, including TCE. In this study, Fischer 344 rats were administered TCE by gavage at 0, 1,125, and 1,500 mg/kg/d on gestation days 6−19, and litters were examined on postnatal days 1, 3, and 6. TCE-related increased incidences of full-litter resorptions, decreased litter sizes, and decreased mean pup birth weights were observed at both treatment levels. Additionally, TCE treatment was reported to be associated with increased incidences of eye abnormalities (microphthalmia or anophthalmia). Increased incidences of fetal loss and percent pups with eye abnormalities were confirmed by Narotsky et al. (1995) in a preliminary dose-setting study that treated Fischer 344 rats with TCE by gavage doses of 475, 633, 844, or 1,125 mg/kg/d on gestation days 6−15, and then in a 5 × 5 × 5 mixtures study that used TCE doses of 0, 10.1, 32, 101, and 320 mg/kg/d on GD 6−15. In both studies, dams were allowed to deliver, and pups were examined postnatally. The incidence of ocular defects observed across all TCE treatment levels tested is presented in Table 4-86.

Other developmental findings in this study included increased full litter resorption at 475, 844, and 1,125 mg/kg/d; increased postnatal mortality at 425 mg/kg/d. Pup body weights were decreased (not significantly) on PND 1 and 6 at 1,125 mg/kg/d. In both the Narotsky and Kavlock (1995) and Narotsky et al. (1995) studies, significantly decreased maternal body weight gain was observed at the same treatment levels at which full litter resorption was noted. Additionally, in Narotsky et al. (1995) maternal observations included delayed parturition at 475, 844, and 1,125 mg/kg/d, ataxia at 633 mg/kg/d, and mortality at 1,125 mg/kg/d.

Cosby and Dukelow (1992) administered TCE in corn oil by gavage to female B6D2F1 mice (28−62/group) on gestation days 1−5, 6−10, or 11−15 (where mating = GD 1). Dose levels were 0, 1/100 and 1/10 of the oral LD_{50} (i.e., 0, 24.02, and 240.2 mg/kg body weight). Dams

were allowed to deliver; litters were evaluated for pup count sex, weight, and crown-rump length until weaning (PND 21). Some litters were retained until 6 weeks of age at which time gonads (from a minimum of 2 litters/group) were removed, weighed, and examined. No treatment-related reproductive or developmental abnormalities were observed.

A single dose of TCE was administered by gavage to pregnant CD-1 mice (9−19/group) at doses of 0, 0.1, or 1.0 μg/kg in distilled water, or 0, 48.3, or 483 mg/kg in olive oil, 24 hours after premating human chorionic gonadotropin (hCG) injection (Coberly et al., 1992). At 53 hours after the hCG-injection, the dams were terminated, and the embryos were flushed from excised oviducts. Chimera embryos were constructed, cultured, and examined. Calculated proliferation ratios did not identify any differences between control and treated blastomeres. A lack of treatment-related adverse outcome was also noted when the TCE was administered by i.p. injection to pregnant mice (16−39/group) at 24 and 48 hours post-hCG at doses of 0, 0.01, 0.02, or 10 μg/kg body weight.

In a study intended to confirm or refute the cardiac teratogenicity of TCE that had been previously observed in chick embryos, Dawson et al. (1990) continuously infused the gravid uterine horns of Sprague-Dawley rats with solutions of 0-, 15-, or 1,500-ppm TCE (or 1.5 or 150-ppm dichloroethylene) on gestation days 7−22. At terminal cesarean section on gestation Day 22, the uterine contents were examined, and fetal hearts were removed and prepared for further dissection and examination under a light microscope. Cardiac malformations were observed in 3% of control fetuses, 9% of the 15-ppm TCE fetuses ($p = 0.18$), and 14% of the 1,500-ppm TCE fetuses. ($p = 0.03$). There was a >60% increase in the percent of defects with a 100-fold increase in dose. No individual malformation or combination of abnormalities was found to be selectively induced by treatment.

To further examine these TCE-induced cardiac malformations in rats, Dawson et al. (1993) administered 0, 1.5 or 1,100-ppm TCE in drinking water to female Sprague-Dawley rats. Experimental treatment regimens were (1) a period of approximately 2 months prior to pregnancy plus the full duration of pregnancy, (2) the full duration of pregnancy only, or (3) an average of 3 months before pregnancy only. The average total daily doses of TCE consumed for each exposure group at both dose levels were

	1.5 ppm	1,100 ppm
Group 1	23.5 μL	1,206 μL
Group 2	0.78 μL	261 μL
Group 3	3.97 μL	1,185 μL

The study also evaluated 0, 0.15, or 110-ppm dichloroethylene in drinking water, with treatment administered (1) two months prior to pregnancy plus the full duration of pregnancy, or (2) an average of 2 months before pregnancy only. At terminal cesarean section, uterine contents were examined, fetuses were evaluated for external defects, and the heart of each fetus was removed for gross histologic examination under a dissecting microscope, conducted without knowledge of treatment group. There were no differences between TCE-treated and control group relative to percentage of live births, implants, and resorptions. The percentage of cardiac defects in TCE-treated groups ranged from 8.2 to 13.0%, and was statistically significant as compared to the control incidence of 3%. The dose-response was relatively flat, even in spite of the extensive difference between the treatment levels. There was a broad representation of various types of cardiac abnormalities identified, notably including multiple transposition, great artery, septal, and valve defects (see Table 4-88). No particular combination of defects or syndrome predominated. Exposure before pregnancy did not appear to be a significant factor in the incidence of cardiac defects.

Table 4-88. Types of congenital cardiac defects observed in TCE-exposed fetuses (Dawson et al., 1993, Table 3)

Cardiac abnormalities	Control	TCE concentrations					
		Premating		Premating/gestation		Gestation only	
		1,100 ppm	1.5 ppm	1,100 ppm	1.5 ppm	1,100 ppm	1.5 ppm
d-transposition (right chest)	2						
l-transposition (left chest)					2		1
Great artery defects				1	2		1
Atrial septal defects	1	7	3	19	5	7	4
Mitral valve defects				5	8		
Tricuspid valve defects		1		1	2		
Ventricular septal defects							
Subaortic	1			4	1	1	2
Membranous				2			
Muscular	2	1	1	4		4	1
Endocardial cushion defect	1					1	
Pulmonary valve defects			3	2	1		1
Aortic valve defects			1	2	2	2	
Situs inversus				1			
Total abnormalities	7	9	8	41	23	15	10
Total abnormal hearts	7	9	8	40	23	11	9

In an attempt to determine a threshold for cardiac anomalies following TCE exposures, Johnson et al. (2003, 2005) compiled and reanalyzed data from five studies conducted from 1989−1995. In these studies, TCE was administered in drinking water to Sprague-Dawley rats throughout gestation (i.e., a total of 22 days) at levels of 2.5 ppb (0.0025 ppm), 250 ppb (0.25-ppm), 1.5, or 1,100 ppm. The dams were terminated on the last day of pregnancy and fetuses were evaluated for abnormalities of the heart and great vessels. The control data from the five studies were combined prior to statistical comparison to the individual treated groups, which were conducted separately. The study author reported that significant increases in the percentage of abnormal hearts and the percentage of litters with abnormal hearts were observed in a generally dose-responsive manner at 250 ppb and greater (see Table 4-89).

Table 4-89. Types of heart malformations per 100 fetuses (Johnson et al., 2003, Table 2, p. 290)

Type of defect/100 fetuses	Control	TCE dose group			
		1,100 ppm	1.5 ppm	250 ppb	2.5 ppb
Abnormal looping	0.33		1		
Coronary artery/sinus				1.82	
Aortic hypoplasia			0.55		
Pulmonary artery hypoplasia			0.55		
Atrial septal defect	1.16	6.67	2.21	0.91	
Mitral valve defect	0.17			0.91	
Tricuspid valve defect				0.91	
Ventricular septal defect					
Perimembranous (subaortic)	0.33	2.86	1.66		
Muscular	0.33	0.95	0.55		
Atriventricular septal defect	0.17	0.95			
Pulmonary valve defect					
Aortic valve defects		1.9		0.91	
Fetuses with abnormal hearts (*n*)	13	11	9	5	0
Total fetuses (*n*)	606	105	181	110	144
Litters with fetuses with abnormal hearts/litter (*n*)	9/55	6/9	5/13	4/9	0/12
Litter with fetuses with abnormal hearts/no. litters (%)	16.4	66.7	38.5	44.4	0.0

In a study by Fisher et al. (2001), pregnant Sprague-Dawley rats were administered daily gavage doses on GD 6−15 of TCE (500 mg/kg/d), TCA (300 mg/kg/d), or DCA (300 mg/kg/d).

Cesarean delivery of fetuses was conducted on GD 21. Water and soybean oil negative control groups, and a retinoic acid positive control group were also conducted simultaneously. Maternal body weight gain was not significantly different from control for any of the treated groups. No significant differences were observed for number of implantations, resorptions, or litter size. Mean fetal body weight was reduced by treatment with TCA and DCA. The incidence of heart malformations was not significantly increased in treated groups as compared to controls. The fetal rate of cardiac malformations ranged from 3 to 5% across the TCE, TCA, and DCA dose groups and from 6.5 to 2.9% for the soybean and water control dose groups, respectively. It was suggested that the apparent differences between the results of this study and the Dawson et al. (1993) study may be related to factors such as differences in purity of test substances or in the rat strains, or differences in experimental design (e.g., oral gavage versus drinking water, exposure only during the period of organogenesis versus during the entire gestation period, or the use of a staining procedure). The rats from this study were also examined for eye malformations to follow-up on the findings of Narotsky (1995). As reported in Warren et al. (2006), gross evaluation of the fetuses as well as computerized morphometry conducted on preserved and sectioned heads revealed no ocular anomalies in the groups treated with TCE. This technique allowed for quantification of the lens area, global area, medial canthus, distance, and interlocular distance. DCA treatment was associated with statistically significant reductions in the lens area, globe area, and interlocular distance. All four measures were reduced in the TCA-treated group, but not significantly. The sensitivity of the assay was demonstrated successfully with the use of a positive control group that was dosed on GD 6−15 with a known ocular teratogen, retinoic acid (15 mg/kg/d).

Johnson et al. (1998a, b) conducted a series of studies to determine whether specific metabolites of TCE or dichloroethylene were responsible for the cardiac malformations observed in rats following administration during the period of organogenesis. Several metabolites of the two chemicals were administered in drinking water to Sprague-Dawley rats from GD 1−22. These included carboxy methylcystine, dichloroacetaldehyde, dichlorovinyl cystine, monochloroacetic acid, trichloroacetic acid, trichloroacetaldehyde, and trichloroethanol. Dichloroacetic acid, a primary common metabolite of TCE and dichloroethylene, was not included in these studies. The level of each metabolite administered in the water was based upon the dosage equivalent expected if 1,100 ppm (the limit of solubility) TCE broke down completely into that metabolite. Cesarean sections were performed on GD 22, uterine contents were examined, and fetuses were processed and evaluated for heart defects according to the procedures used by Dawson et al. (1993). No treatment-related maternal toxicity was observed for any metabolite group. Adverse fetal outcomes were limited to significantly increased incidences of fetuses with abnormal hearts (see Table 4-90). Significant increases in fetuses with

cardiac defects (on a per-fetus and per-litter basis) were observed for only one of the metabolites evaluated, i.e., trichloroacetic acid (2,730 ppm, equivalent to a dose of 291 mg/kg/d). Notably, significant increases in fetuses with cardiac malformations were also observed with 1.5 or 1,100-ppm TCE (0.218 or 129 mg/kg/d), or with 0.15 or 110-ppm DCE (0.015 or 10.64 mg/kg/d), but in each case only with prepregnancy-plus-pregnancy treatment regimens. The cardiac abnormalities observed were diverse and did not segregate to any particular anomaly or grouping. Dose related increases in response were observed for the overall number of fetuses with any cardiac malformation for both TCE and DCE; however, no dose-related increase occurred for any specific cardiac anomaly (Johnson et al., 1998b).

The TCE metabolites TCA and DCA were also studied by Smith et al. (1989, 1992). Doses of 0, 330, 800, 1,200, or 1,800 mg/kg TCA were administered daily by oral gavage to Long-Evan hooded rats on gestation days 6−15. Similarly, DCA was administered daily by gavage to Long-Evans rats on GD 6−15 in two separate studies, at 0, 900, 1,400, 1,900, or 2,400 mg/kg/d and 0, 14, 140, or 400 mg/kg/d. Embryo lethality and statistically or biologically significant incidences of orbital anomalies (combined soft tissue and skeletal findings) were observed for TCA at ≥800 mg/kg/d, and for DCA at ≥900 mg/kg/d. Fetal growth (body weight and crown-rump length) was affected at ≥330 mg/kg/d for TCE and at ≥400 mg/kg/d for DCA. For TCA, the most common cardiac malformations observed were levocardia at ≥330 mg/kg/d and interventricular septal defect at ≥800 mg/kg/d. For DCA, levocardia was observed at ≥900 mg/kg/d, interventricular septal defect was observed at ≥1,400 mg/kg/d, and a defect between the ascending aorta and right ventricle was observed in all treated groups (i.e., ≥14 mg/kg/d, although the authors appeared to discount the single fetal finding at the lowest dose tested). Thus, NOAELs were not definitively established for either metabolite, although it appears that TCA was generally more potent than DCA in inducing cardiac abnormalities.

Table 4-90. Congenital cardiac malformations (Johnson et al., 1998b, Table 2, p. 997)

Heart abnormalities	Treatment group												
	Normal water	TCE p+p 1,100 ppm	TCE p+p 1.5 ppm	TCE p 1,100 ppm	DCE p+p 110 ppm	DCE p+p 0.15 ppm	TCAA p 2,730 ppm	MCAA p 1,570 ppm	TCEth p 1,249 ppm	TCAld p 1,232 ppm	DCAld p 174 ppm	CMC p 473 ppm	DCVC p 50 ppm
Abnormal looping	2	-	2	-	-	-	-	-	-	-	-	-	-
Aortic hypoplasia	-	1	1	-	1	-	1	-	1	-	1	-	1
Pulmonary artery hypoplasia	-	-	1	-	-	-	2	1	-	-	2	-	-
Atrial septal defects	7	19	5	7	11	7	3	3	-	2	-	-	1
Mitral valve defects, hypoplasia or ectasia	1	5	8	-	4	3	1	-	1	2	-	-	1
Tricuspid valve defects, hypoplasia or ectasia	-	1	1	-	1	-	-	-	1	-	-	-	-
Ventricular septal defects													
Perimembranous [a]	2	6	2	1	4	1	4	-	-	3	-	1	-
Muscular	2	4	-	4	2	1	1	-	1	-	-	2	2
Atrioventricual septal defects	1	-	-	1	1	-	-	-	-	-	-	-	-
Pulmonary valve defects	-	2	1	-	1	-	1	3	1	1	-	-	-
Aortic valve defects	-	2	2	2	2	3	-	-	1	-	-	1	-
Situs inversus	-	1	-	-	-	-	-	-	-	-	-	-	-
Total													
Abnormal hearts	15	41	23	15	25	15	13	7	6	8	3	4	5
Fetuses with abnormal hearts	13	40*	22*	11*	24*	14*	12*	6	5	8	3	4	5
Fetuses	605	434	255	105	184	121	114	132	121	248	101	85	140

[a]Subaortic.
[b]Per-fetus statistical significance (Fisher exact test).

p+p = pregnancy and prepregnancy, p = pregnancy.

These findings were followed up by a series of studies on DCA reported by Epstein et al. (1992), which were designed to determine the most sensitive period of development and further characterize the heart defects. In these studies, Long-Evans hooded rats were dosed by oral gavage with a single dose of 2,400 mg/kg/d on selected days of gestation (6−8, 9−11, or 12−15); with a single dose of 2,400 mg/kg on Days 10, 11, 12, or 13; or with a single dose of 3,500 mg/kg on Days 9, 10, 11, 12, or 13. The heart defects observed in these studies were diagnosed as high interventricular septal defects rather than membranous type interventricular septal defects. The authors hypothesized that high intraventricular septal defects are a specific type of defect produced by a failure of proliferating interventricular septal tissue to fuse with the right tubercle of the atrioventricular cushion tissue. This study identified gestation days 9 through 12 as a particularly sensitive period for eliciting high interventricular septal defects. It was postulated that DCA interferes with the closure of the tertiary interventricular foramen, allowing the aorta to retain its embryonic connection with the right ventricle. Further, it was suggested that the selectivity of DCA in inducing cardiac malformations may be due to the disruption of a discrete cell population.

TCE and its metabolites DCE and TCAA were administered in drinking water to pregnant Sprague-Dawley rats from gestation days 0−11 (Collier et al., 2003). Treatment levels were 0, 110, or 1,100 ppm (i.e., 0, 830 or 8,300 μgM) TCE; 0, 11, or 110 ppm (i.e., 0, 110, or 1,100 μgM) DCE; 0, 2.75, or 27.3 mg/mL (i.e., 0, 10, or 100 mM) TCAA. Embryos (including hearts) were harvested between embryonic days 10.5−11, since this is the stage at which the developmental processes of myoblast differentiation, cardiac looping, atrioventricular valve formation, and trabeculation would typically be occurring. A PCR based subtraction scheme was used to identify genes that were differentially regulated with TCE or metabolite exposure. Numerous differentially regulated gene sequences were identified. Up-regulated transcripts included genes associated with stress response (Hsp 70) and homeostasis (several ribosomal proteins). Down-regulated transcripts included extracellular matrix components (GPI-p137 and vimentin) and Ca^{2+} responsive proteins (Serca-2 Ca^{2+}-ATPase and β–catenin). Serca-2 Ca^{2+} and GPI-p137 were identified as two possible markers for fetal TCE exposure. Differential regulation of expression of these markers by TCE was confirmed by dot blot analysis and semiquantitative real time PCR with decreased expression seen at levels of TCE exposure between 100 and 250 ppb (0.76 and 1.9 μM).

4.8.3.2.1.2.1. *Developmental neurotoxicity and developmental immunotoxicity.* Several studies were conducted that included assessments of the effects of TCE oral exposure on the developing nervous system (Fredriksson et al., 1993; Isaacson and Taylor, 1989; Noland-Gerbec et al., 1986; George et al., 1986; Dorfmueller et al., 1979; Blossom et al., 2008) or immune system (Peden-

Adams et al., 2006, 2008; Blossom and Doss, 2007; Blossom et al., 2008). These studies, summarized below, are addressed in additional detail in Section 4.3 (nervous system) and Section 4.6.2.1.2 (immune system).

4.8.3.2.1.2.2. *Developmental neurotoxicity.* Fredriksson et al. (1993) conducted a study in male NMRI weanling mice (12/group, selected from 3−4 litters), which were exposed to trichloroethylene by oral gavage at doses of 0 (vehicle), 50, or 290 mg/kg/d TCE in a fat emulsion vehicle, on PNDs 10−16. Locomotor behavior (horizontal movement, rearing and total activity) were assessed over three 20-minute time periods at postnatal days 17 and 60. There were no effects of treatment in locomotor activity at PND 17. At PND 60, the mice treated with 50 and 290 mg/kg/d TCE showed a significant ($p < 0.01$) decrease in rearing behavior at the 0−20 and 20−40 minute time points, but not at the 40−60 minute time point. Mean rearing counts were decreased by over 50% in treated groups as compared to control. Horizontal activity and total activity were not affected by treatment.

Open field testing was conducted in control and high-dose F1 weanling Fischer 344 rat pups in an NTP reproduction and fertility study with continuous breeding (George et al., 1986). In this study, TCE was administered at dietary levels of 0, 0.15, 0.30, or 0.60%. The open field testing revealed a significant ($p < 0.05$) dose-related trend toward an increase in the time required for male and female pups to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment.

Taylor et al. (1985) administered TCE in drinking water (0, 312, 625, or 1,250 ppm) to female Sprague-Dawley rats for 14 days prior to breeding, and from gestation Day 0 through offspring postnatal Day 21. The number of litters/group was not reported, nor did the study state how many pups per litter were evaluated for behavioral parameters. Exploratory behavior was measured in the pups in an automated apparatus during a 15-minute sampling period on PND 28, 60, and 90. Additionally, wheel-running, feeding, and drinking behavior was monitored 24 hours/day on PND 55-60. The number of exploratory events was significantly increased by approximately 25−50% in 60- and 90-day old male TCE-treated rats at all dose levels, with the largest effect observed at the highest dose level tested, although there were no effects of treatment on the number of infrared beam-breaks. No difference between control and treated rats was noted for pups tested on PND 28. Wheel-running activity was increased approximately 40% in 60-day old males exposed to 1,25-ppm TCE as compared to controls. It is notable that adverse outcomes reported in the developmentally-exposed offspring on this study were observed long after treatment ceased.

Using a similar treatment protocol, the effects of TCE on development of myelinated axons in the hippocampus was evaluated by Isaacson and Taylor (1989) in Sprague-Dawley rats.

Female rats (6/group) were exposed in the drinking water from 14 days prior to breeding and through the mating period; then the dams and their pups were exposed throughout the prenatal period and until PND 21, when they were sacrificed. The dams received 0, 312 or 625 ppm (0, 4, or 8.1 mg/day TCE in the drinking water. Myelinated fibers were counted in the hippocampus of 2−3 pups per treatment group at PND 21, revealing a decrease of approximately 40% in myelinated fibers in the CA1 area of the hippocampus of pups from dams at both treatment levels, with no dose-response relationship. There was no effect of TCE treatment on myelination in several other brain regions including the internal capsule, optic tract or fornix.

A study by Noland-Gerbec et al. (1986) examined the effect of pre- and perinatal exposure to TCE on 2-deoxyglucose (2-DG) uptake in the cerebellum, hippocampus and whole brain of neonatal rats. Sprague-Dawley female rats (9−11/group) were exposed via drinking water to 0 or 312 mg TCE/liter distilled water from 14 days prior to mating until their pups were euthanized at postnatal Day 21. The total TCE dose received by the dams was 825 mg over the 61-day exposure period. Pairs of male neonates were euthanized on PND 7, 11, 16, and 21. There was no significant impairment in neonatal weight or brain weight attributable to treatment, nor were other overt effects observed. 2-DG uptake was significantly reduced from control values in neonatal whole brain (9−11%) and cerebellum (8−16%) from treated rats at all ages studied, and hippocampal 2-DG uptake was significantly reduced (7−21% from control) in treated rats at all ages except at PND 21.

In a study by Blossom et al. (2008), MRL +/+ mice were treated in the drinking water with 0 or 0.1 mg/mL TCE from maternal GD 0 through offspring PND 42. Based on drinking water consumption data, average maternal doses of TCE were 25.7 mg/kg/d, and average offspring (PND 24−42) doses of TCE were 31.0 mg/kg/d. In this study, a subset of offspring (3 randomly selected neonates from each litter) was evaluated for righting reflex on PNDs 6, 8, and 10; bar-holding ability on PNDs 15 and 17; and negative geotaxis on PNDs 15 and 17; none of these were impaired by treatment. In an assessment of offspring nest building on PND 35, there was a significant association between impaired nest quality and TCE exposure; however, TCE exposure did not have an effect on the ability of the mice to detect social and nonsocial odors on PND 29 using olfactory habituation and dishabituation methods. Resident intruder testing conducted on PND 40 to evaluate social behaviors identified significantly more aggressive activities (i.e., wrestling and biting) in TCE-exposed juvenile male mice as compared to controls. Cerebellar tissue homogenates from the male TCE-treated mice had significantly lower GSH levels and GSH:oxidized GSH (GSH:GSSG) ratios, indicating increased oxidative stress and impaired thiol status; these have been previously reported to be associated with aggressive behaviors (Franco et al., 2006). Qualitative histopathological examination of the brain did not identify alterations indicative of neuronal damage or inflammation. Although the

study author attempted to link the treatment-related alterations in social behaviors to the potential for developmental exposures to TCE to result in autism in humans, this association is not supported by data and is considered speculative at this time.

As previously noted, postnatal behavioral studies conducted by Dorfmueller et al. (1979) did not identify any changes in general motor activity measurements of rat offspring on PND 10, 20, and 100 following maternal gestational inhalation exposure to TCE at 1,800 ± 200 ppm.

4.8.3.2.1.2.3. *Developmental immunotoxicity.* Peden-Adams et al. (2006) assessed the potential for developmental immunotoxicity following TCE exposures. In this study, B6C3F1 mice (5/sex/group) were administered TCE via drinking water at dose levels of 0, 1,400 or 14,000 ppb from maternal gestation Day 0 to either postnatal 3 or 8, when offspring lymphocyte proliferation, NK cell activity, SRBC-specific IgM production (PFC response), splenic B220+ cells, and thymus and spleen T-cell immunophenotypes were assessed. (A total of 5−7 pups per group were evaluated at Week 3, and the remainder were evaluated at Week 8.) Observed positive responses consisted of suppressed PFC responses in males at both ages and both TCE treatment levels, and in females at both ages at 14,000 ppb and at 8 weeks of age at 1,400 ppb. Spleen numbers of B220+ cells were decreased in 3-week old pups at 14,000 ppb. Pronounced increases in all thymus T-cell subpopulations (CD4+, CD8+, CD4+/CD8+, and CD4-/CD8-) were observed at 8-weeks of age. Delayed hypersensitivity response, assessed in offspring at 8 weeks of age, was increased in females at both treatment levels and in males at 14,000 ppb only. No treatment-related increase in serum anti-dsDNA antibody levels was found in the offspring at 8 weeks of age.

In a study by Blossom and Doss (2007), TCE was administered to groups of pregnant MRL +/+ mice in drinking water at levels of 0, 0.5 or 2.5 mg/mL. TCE was continuously administered to the offspring until young adulthood (i.e., 7−8 weeks of age). Offspring postweaning body weights were significantly decreased in both treated groups. Decreased spleen cellularity and reduced numbers of CD4+, CD8+, and B220+ lymphocyte subpopulations were observed in the postweaning offspring. Thymocyte development was altered by TCE exposures (significant alterations in the proportions of double-negative subpopulations and inhibition of *in vitro* apoptosis in immature thymocytes). A dose-dependent increase in CD4+ and CD8+ T-lymphocyte IFNγ was observed in peripheral blood by 4−5 weeks of age, although these effects were no longer observed at 7−8 weeks of age. Serum anti-histone autoantibodies and total IgG_{2a} were significantly increased in treated offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys at sacrifice.

Blossom et al. (2008) administered TCE to MRL +/+ mice (8 dams/group) in the drinking water at levels of 0 or 0.1 mg/mL from GD 0 through offspring postnatal Day 42. Average

maternal doses of TCE were 25.7 mg/kg/d, and average offspring (PND 24−42) doses of TCE were 31.0 mg/kg/d. Subsets of offspring were sacrificed at PND 10 and 20, and thymus endpoints (i.e., total cellularity, CD4+/CD8+ ratios, CD24 differentiation markers, and double-negative subpopulation counts) were evaluated. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at PND 20 (sexes combined). TCE treatment also appeared to promote T-cell differentiation and maturation at PND 42. Indicators of oxidative stress were measured in the thymus at PND 10 and 20, and in the brain at PND 42,.and *ex vivo* evaluation of cultured thymocytes indicated increased ROS generation. Mitogen-induced intracellular cytokine production by splenic CD4+ and CD8+ T-cells was evaluated in juvenile mice and brain tissue was examined at PND 42 for evidence of inflammation. Evaluation of peripheral blood indicated that splenic CD4+ T-cells from TCE-exposed PND 42 mice produced significantly greater levels of IFN-γ and IL-2 in males and TNF-α in both sexes. There was no effect on cytokine production on PND 10 or 20.

Peden-Adams et al. (2008) administered TCE to MRL+/+ mice (unspecified number of dams/group) in drinking water at levels of 0, 1,400, or 14,000 ppb from GD 0 and continuing until the offspring were 12 months of age. At 12 months of age, final body weight; spleen, thymus, and kidney weights; spleen and thymus lymphocyte immunophenotyping (CD4 or CD8); splenic B-cell counts; mitogen-induced splenic lymphocyte proliferation; serum levels of autoantibodies to dsDNA and GA, periodically measured from 4 to 12 months of age; and urinary protein measures were recorded. Reported sample sizes for the offspring measurements varied from 6 to 10 per sex per group; the number of source litters represented within each sample was not specified. The only organ weight alteration was an 18% increase in kidney weight in the 1,400 ppb males. Splenic CD4-/CD8- cells were altered in female mice (but not males) at 1,400 ppm only. Splenic T-cell populations, numbers of B220+ cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8+, CD4-/CD8-, and CD4+) were significantly decreased in male but not female mice following exposure to 14,000 ppb TCE, and CD4+/CD8+ cells were significantly reduced in males by treatment with both TCE concentrations. Autoantibody levels (anti-dsDNA and anti-GA) were not increased in the offspring over the course of the study.

Although all of the developmental immunotoxicity studies with TCE (Peden-Adams et al., 2006, 2008; Blossom and Doss, 2007; Blossom et al., 2008) exposed the offspring during critical periods of pre- and postnatal immune system development, they were not designed to assess issues such as post-treatment recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures.

4.8.3.2.1.3. ***Intraperitoneal exposures.*** The effect of TCE on pulmonary development was evaluated in a study by Das and Scott (1994). Pregnant Swiss-Webster mice (5/group) were administered a single intraperitoneal injection of TCE in peanut oil at doses of 0 or 3,000 mg/kg on gestation Day 17 (where mating = Day 1). Lungs from GD 18 and 19 fetuses and from neonates on PND 1, 5, and 10 were evaluated for phospholipid content, DNA, and microscopic pathology. Fetal and neonatal (PND 1) mortality was significantly increased ($p < 0.01$) in the treated group. Pup body weight and absolute lung weight were significantly decreased ($p < 0.05$) on PND 1, and mean absolute and relative (to body weight) lung weights were significantly decreased on GD 18 and 19. Total DNA content (μg/mg lung) was similar between control and treated mice, but lung phospholipid was significantly ($p < 0.05$) reduced on GD 19 and significantly increased ($p < 0.05$) on PND 10 in the TCE-treated group. Microscopic examination revealed delays in progressive lung morphological development in treated offspring, first observed at GD 19 and continuing at least through PND 5.

4.8.3.2.2. ***Studies in nonmammalian species.***

4.8.3.2.2.1. ***Avian.*** Injection of White Leghorn chick embryos with 1, 5, 10, or 25 μmol TCE per egg on Days 1 and 2 of embryogenesis demonstrated mortality, growth defects, and morphological anomalies at evaluation on Day 14 (Bross et al., 1983). These findings were consistent with a previous study that had been conducted by Elovaara et al. (1979). Up to 67% mortality was observed in the treated groups, and most of the surviving embryos were malformed (as compared to a complete absence of malformed chicks in the untreated and mineral-oil-treated control groups). Reported anomalies included subcutaneous edema, evisceration (gastroschisis), light dermal pigmentation, beak malformations, club foot, and patchy feathering. Retarded growth was observed as significantly ($p < 0.05$) reduced crown-rump, leg, wing, toe, and beak lengths as compared to untreated controls. This study did not identify any liver damage or cardiac anomalies.

In a study by Loeber et al. (1988), 5, 10, 15, 20, or 25 μmol TCE was injected into the air space of White Longhorn eggs at embryonic stages 6, 12, 18, or 23. Embryo cardiac development was examined in surviving chicks in a double-blinded manner at stages 29, 34, or 44. Cardiac malformations were found in 7.3% of TCE-treated hearts, compared to 2.3% of saline controls and 1.5% of mineral oil controls. The observed defects included septal defects, cor biloculare, conotruncal abnormalities, atrioventricular canal defects, and abnormal cardiac muscle.

Drake et al. (2006a) injected embryonated White Leghorn chicken eggs (Babcock or Bovan strains) with 0, 0.4, 8, or 400 ppb TCE per egg during the period of cardiac valvuloseptal morphogenesis (i.e., 2−3.3 days incubation). The injections were administered in four aliquots at

Hamberger and Hamilton (HH) stages 13, 15, 17, and 20, which spanned the major events of cardiac cushion formation, from induction through mesenchyme transformation and migration. Embryos were harvested 22 hours after the last injection (i.e., HH 24 or HH 30) and evaluated for embryonic survival, apoptosis, cellularity and proliferation, or cardiac function. Survival was significantly reduced for embryos at 8 and 400 ppb TCE at HH 30. Cellular morphology of cushion mesenchyme, cardiomyocytes, and endocardiocytes was not affected by TCE treatment; however, the proliferative index was significantly increased in the atrioventricular canal (AVC) cushions at both treatment levels and in the outflow tract (OFT) cushions at 8 ppb. This resulted in significant cushion hypercellularity for both the OFT and AVC of TCE-treated embryos. Similar outcomes were observed in embryos when TCA or TCOH was administered, and the effects of TCA were more severe than for TCE. Doppler ultrasound assessment of cardiac hemodynamics revealed no effects of TCE exposure on cardiac cycle length or heart rate; however, there was a reduction in dorsal aortic blood flow, which was attributed to a 30.5% reduction in the active component of atrioventricular blood flow. Additionally the passive-to-active atrioventricular blood flow was significantly increased in treated embryos, and there was a trend toward lower stroke volume. The overall conclusion was that exposure to 8 ppb TCE during cushion morphogenesis reduced the cardiac output of the embryos in this study. The findings of cardiac malformations and/or mortality following *in ovo* exposure to chick embryos with 8 ppb TCE during the period of valvuloseptal morphogenesis has also been confirmed by Rufer et al. (2008).

In a follow-up study, Drake et al. (2006b) injected embryonated White Leghorn chicken eggs with TCE or TCA during the critical window of avian heart development, beginning at HH stage 3+ when the primary heart field is specified in the primitive streak and ending approximately 50 hours later at HH stage 17, at the onset of chambering. Total dosages of 0, 0.2, 2, 4, 20, or 200 nmol (equivalent to 0, 0.4, 4, 8, 40, or 400 ppb) were injected in four aliquots into each egg yolk during this window (i.e., at stages 3+, 6, 13, and 17: hours 16, 24, 46, and 68). Embryos were harvested at 72 hours, 3.5 days, 4 days or 4.25 days (HH stages 18, 21, 23, or 24, respectively) and evaluated for embryonic survival, cardiac function, or cellular parameters. Doppler ultrasound technology was utilized to assess cardiovascular effects at HH 18, 21, and 23. In contrast with the results of Drake et al. (2006a), all of the functional parameters assessed (i.e., cardiac cycle length, heart rate, stroke volume, and dorsal aortic and atrioventricular blood flow) were similar between control and TCE- or TCA-treated embryos. The authors attributed this difference in response between studies to dependence upon developmental stage at the time of exposure. In this case, the chick embryo was relatively resistant to TCE when exposure occurred during early cardiogenic stages, but was extremely vulnerable when TCE exposure occurred during valvuloseptal morphogenesis. It was opined that this could explain why some

researchers have observed no developmental cardiac effects after TCE exposure to mammalian models, while others have reported positive associations.

4.8.3.2.2.2. ***Amphibian*.** The developmental toxicity of TCE was evaluated in the Frog Embryo Teratogenesis Assay: *Xenopus* by Fort et al. (1991, 1993). Late *Xenopus laevis* blastulae were exposed to TCE, with and without exogenous metabolic activation systems, or to TCE metabolites (dichloroacetic acid, trichloroacetic acid, trichloroethanol, or oxalic acid), and developmental toxicity ensued. Findings included alterations in embryo growth, and increased types and severity of induced malformations. Findings included cardiac malformations that were reportedly similar to those that had been observed in avian studies. It was suggested that a mixed function oxidase-mediated reactive epoxide intermediate (i.e., TCE-oxide) may play a significant role in observed developmental toxicity in *in vitro* tests.

Likewise, McDaniel et al. (2004) observed dose-dependent increases in developmental abnormalities in embryos of four North American amphibian species (wood frogs, green frogs, American toads, and spotted salamanders) following 96-hour exposures to TCE. Median effective concentrations (EC_{50}) for malformations was 40 mg/L for TCE in green frogs, while American toads were less sensitive (with no EC_{50} at the highest concentration tested—85 mg/L). Although significant mortality was not observed, the types of malformations noted would be expected to compromise survival in an environmental context.

4.8.3.2.2.3. ***Invertebrate*.** The response of the daphnid *Ceriodaphnia dubia* to six industrial chemicals, including TCE, was evaluated by Niederlehner et al. (1998). Exposures were conducted for 6−7 days, according to standard U.S. EPA testing guidelines. Lethality, impairment of reproduction, and behavioral changes, such as narcosis and abnormal movement, were observed with TCE exposures. The reproductive sublethal effect concentration value for TCE was found to be 82 μM.

4.8.3.2.3. ***In vitro studies.*** Rat whole embryo cultures were used by Saillenfait et al. (1995) to evaluate the embryotoxicity of TCE, tetrachloroethylene, and four metabolites (trichloroacetic acid, dichloroacetic acid, chloral hydrate, and trichloroacetyl chloride). In this study, explanted embryos of Sprague-Dawley rats were cultured in the presence of the test chemicals for 46 hours and subsequently evaluated. Concentration-dependant decreases in growth and differentiation, and increases in the incidence of morphologically abnormal embryos were observed for TCE at ≥5 mM.

Whole embryo cultures were also utilized by Hunter et al. (1996) in evaluating the embryotoxic potential of a number of disinfection by-products, including the TCE metabolites DCA and TCA. CD-1 mouse conceptuses (GD 9; 3−6 somites) were cultured for 24−26 hours in

treated medium. DCA levels assessed were 0, 734, 1,468, 4,403, 5,871, 7,339, 11,010, or 14,680 μM; TCA levels assessed were 0, 500, 1,000, 2,000, 3,000, 4,000, 5,000 μM. For DCA, neural tube defects were observed at levels of ≥5,871 μM, heart defects were observed at ≥7,339 μM, and eye defects were observed at levels of ≥11,010 μM. For TCA, neural tube defects were observed at levels of ≥2,000 μM, heart and eye defects were observed at ≥3,000 μM. The heart defects for TCA were reported to include incomplete looping, a reduction in the length of the heart beyond the bulboventricular fold, and a marked reduction in the caliber of the heart tube lumen. Overall benchmark concentrations (i.e., the lower limit of the 95% confidence interval required to produce a 5% increase in the number of embryos with neural tube defects) were 2,451.9 μM for DCA and 1,335.8 μM for TCA (Richard and Hunter, 1996).

Boyer et al. (2000) used an *in vitro* chick-atrioventricular (AV) canal culture to test the hypothesis that TCE might cause cardiac valve and septal defects by specifically perturbing epithelial-mesenchymal cell transformation of endothelial cells in the AV canal and outflow tract areas of the heart. AV explants from Stage 16 White Leghorn chick embryos were placed in hydrated collagen gels, with medium and TCE concentrations of 0, 50, 100, 150, 200, or 250 ppm. TCE was found to block the endothelial cell-cell separation process that is associated with endothelial activation as well as to inhibit mesenchymal cell formation across all TCE concentrations tested. TCE did not, however, have an effect on the cell migration rate of fully formed mesenchymal cells. TCE-treatment was also found to inhibit the expression of transformation factor Mox-1 and extracellular matrix protein fibrillin 2, two protein markers of epithelial-mesenchyme cell transformation.

4.8.3.3. *Discussion/Synthesis of Developmental Data*

In summary, an overall review of the weight of evidence in humans and experimental animals is suggestive of the potential for developmental toxicity with TCE exposure. A number of developmental outcomes have been observed in the animal toxicity and the epidemiological data, as discussed below. These include adverse fetal/birth outcomes including death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, small for gestational age, intrauterine growth restriction, decreased postnatal growth), and congenital malformations, in particular cardiac defects. Postnatal developmental outcomes include developmental neurotoxicity, developmental immunotoxicity, and childhood cancer.

4.8.3.3.1. ***Adverse fetal and early neonatal outcomes.*** Studies that demonstrate adverse fetal or early neonatal outcomes are summarized in Table 4-91. In human studies of prenatal TCE exposure, increased risk of spontaneous abortion was observed in some studies (ATSDR, 2001;

Taskinen et al., 1994; Windham et al., 1991), but not in others (ATSDR, 2001, 2008; Goldberg et al., 1990; Lagakos et al., 1986; Lindbohm et al., 1990; Taskinen et al., 1989). In addition, perinatal deaths were observed after 1970, but not before 1970 (Lagakos et al., 1986). In rodent studies that examined offspring viability and survival, there was an indication that TCE exposure may have resulted in increased pre-and/or postimplantation loss (Kumar et al., 2000a; Healy et al., 1982; Narotsky and Kavlock, 1995), and in reductions in live pups born as well as in postnatal and postweaning survival (George et al., 1985, 1986).

Table 4-91. Summary of adverse fetal and early neonatal outcomes associated with TCE exposures

Positive finding	Species	Citation
Spontaneous abortion, miscarriage, pre-and/or postimplantation loss	Human	ATSDR, 2001[a] Taskinen et al., 1994[a] Windham et al., 1991
	Rat	Kumar et al., 2000a Healy et al., 1982 Narotsky and Kavlock, 1995 Narotsky et al., 1995
Perinatal death, reduction in live births	Human	Lagakos et al., 1986[b]
	Mouse	George et al., 1985
	Rat	George et al., 1986
Postnatal and postweaning survival	Mouse	George et al., 1985
	Rat	George et al., 1986
Decreased birth weight, small for gestational age, postnatal growth	Human	ATSDR, 1998 ATSDR, 2006 Rodenbeck et al., 2000[c] Windham et al., 1991
	Mouse	George et al., 1985
	Rat	George et al., 1986 Healy et al., 1982 Narotsky and Kavlock, 1995 Narotsky et al., 1995

[a]Not significant.
[b]Observed for exposures after 1970, but not before.
[c]Increased risk for very low birth weight but not low birth weight or full-term low birth weight.

Decreased birth weight and small for gestational age was observed (ATSDR, 1998, 2006; Rodenbeck et al., 2000; Windham et al., 1991), however, no association was observed in other

studies (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). While comprising both occupational and environmental exposures, these human studies are overall not highly informative due to their small numbers of cases and limited exposure characterization or to the fact that exposures to mixed solvents were involved. However, decreased fetal weight, live birth weights and postnatal growth were also observed in rodents (George et al., 1985, 1986; Healy et al., 1982; Narotsky and Kavlock, 1995), adding to the weight of evidence for this endpoint. It is noted that the rat studies reporting effects on fetal or neonatal viability and growth used Fischer 344 or Wistar rats, while several other studies, which used Sprague-Dawley rats, reported no increased risk in these developmental measures (Carney et al., 2006; Hardin et al., 1981; Schwetz et al., 1975).

Overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

4.8.3.3.2. ***Cardiac malformations.*** A discrete number of epidemiological studies and studies in laboratory animal models have identified an association between TCE exposures and cardiac defects in developing embryos and/or fetuses. These are listed in Table 4-92. Additionally, a number of avian and rodent in vivo studies and in vitro assays have examined various aspects of the induction of cardiac malformations.

In humans, an increased risk of cardiac defects has been observed after exposure to TCE in studies reported by ATSDR (2006, 2008) and Yauck et al. (2004), although others saw no significant effect (Bove et al., 1995; Bove, 1996; Goldberg et al., 1990; Lagakos et al., 1986), possibly due to a small number of cases. In addition, altered heart rate was seen in one study (Jasinka, 1965, translation). A cohort of water contamination in Santa Clara County, California is often cited as a study of TCE exposure and cardiac defects; however, the chemical of exposure is in fact trichloroethane, not TCE (Deane et al., 1989; Swan et al., 1989).

In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development. As described in Section 4.8.2.2.1, cardiac malformations have been reported in chick embryos exposed to TCE (Bross et al., 1983; Loeber et al., 1988; Boyer et al., 2000; Drake et al., 2006a, b; Mishima et al., 2006; Rufer et al., 2008). Additionally, a number of studies were conducted in rodents in which cardiac malformations were observed in fetuses following the oral administration of TCE to maternal animals during gestation (Dawson et al., 1990, 1993; Johnson et al., 2003, 2005; see Section 4.8.2.2.1.2). Cardiac defects were also observed in rats following oral gestational treatment with metabolites of TCE (Johnson et al., 1998a, b; Smith et al., 1989, 1992; Epstein et al., 1992).

Table 4-92. Summary of studies that identified cardiac malformations associated with TCE exposures

Finding	Species	Citations
Cardiac defects	Human	ATSDR, 2006, 2008; Yauck et al., 2004;
	Rat	Dawson et al., 1990, 1993 Johnson et al., 2003, 2005 Johnson et al., 1998a, b* Smith et al., 1989,* 1992* Epstein et al., 1992*
	Chicken	Bross et al., 1983 Boyer et al., 2000 Loeber et al., 1988 Drake et al., 2006a, b Mishima et al., 2006 Rufer et al., 2008
Altered heart rate	Human	Jasinka, 1965, translation

*Metabolites of TCE.

However, cardiac malformations were not observed in a number of other studies in laboratory animals in which TCE was administered during the period of cardiac organogenesis and fetal visceral findings were assessed. These included inhalation studies in rats (Dorfmueller et al., 1979; Schwetz et al., 1975; Hardin et al., 1981; Healy et al., 1982; Carney et al., 2006) and rabbits (Hardin et al., 1981), and oral gavage studies in rats (Narotsky et al., 1995; Narotsky and Kavlock, 1995; Fisher et al., 2001) and mice (Cosby and Dukelow, 1992).

It is generally recognized that response variability among developmental bioassays conducted with the same chemical agent may be related to factors such as the study design (e.g., the species and strain of laboratory animal model used, the day(s) or time of day of dose administration in relation to critical developmental windows, the route of exposure, the vehicle used, the day of study termination), or the study methodologies (e.g., how fetuses were processed, fixed, and examined; what standard procedures were used in the evaluation of morphological landmarks or anomalies, and whether there was consistency in the fetal evaluations that were conducted). In the case of studies that addressed cardiac malformations, there is additional concern as to whether detailed visceral observations were conducted, whether or not cardiac evaluation was conducted using standardized dissection procedures (e.g., with the use of a dissection microscope or including confirmation by histopathological evaluation, and whether the examinations were conducted by technicians who were trained and familiar with

fetal cardiac anatomy). Furthermore, interpretation of the findings can be influenced by the analytical approaches applied to the data as well as by biological considerations such as the historical incidence data for the species and strain of interest. These issues have been critically examined in the case of the TCE developmental toxicity studies (Hardin et al., 2005; Watson et al., 2006).

In the available animal developmental studies with TCE, differences were noted in the procedures used to evaluate fetal cardiac morphology following TCE gestational exposures across studies, and some of these differences may have resulted in inconsistent fetal outcomes and/or the inability to detect cardiac malformations. Most of the studies that did not identify cardiac anomalies used a traditional free-hand sectioning technique (as described in Wilson, 1965) on fixed fetal specimens (Dorfmueller et al., 1979; Schwetz et al., 1975; Hardin et al., 1981; Healy et al., 1982). Detection of cardiac anomalies can be enhanced through the use of a fresh dissection technique as described by Staples (1974) and Stuckhardt and Poppe (1984); a significant increase in treatment-related cardiac heart defects was observed by Dawson et al. (1990) when this technique was used. Further refinement of this fresh dissection technique was employed by Dawson and colleagues at the University of Arizona (UA), resulting in several additional studies that reported cardiac malformations (Dawson et al., 1993; Johnson et al., 2003, 2005). However, two studies conducted in an attempt to verify the teratogenic outcomes of the UA laboratory studies used the same or similar enhanced fresh dissection techniques and were unable to detect cardiac anomalies (Fisher et al., 2001; Carney et al., 2001). Although the Carney et al. study was administered via inhalation (a route which has not previously been shown to produce positive outcomes), the Fisher et al. study was administered orally and included collaboration between industry and UA scientists. It was suggested that the apparent differences between the results of the Fisher et al. study and the Dawson et al. (1993) and Johnson et al. studies may be related to factors such as differences in purity of test substances or in the rat strains, or differences in experimental design (e.g., oral gavage versus drinking water, exposure only during the period of organogenesis versus during the entire gestation period, or the use of a staining procedure).

It is notable that all studies that identified cardiac anomalies following gestational exposure to TCE or its metabolites were (1) conducted in rats and (2) dosed by an oral route of exposure (gavage or drinking water). Cross-species and route-specific differences in fetal response may be due in part to toxicokinetic factors. Although a strong accumulation and retention of TCA was found in the amniotic fluid of pregnant mice following inhalation exposures to TCE (Ghantous et al., 1986), other toxicokinetic factors may be critical. The consideration of toxicokinetics in determining the relevance of murine developmental data for human risk assessment is briefly discussed by Watson et al. (2006). There are differences in the

metabolism of TCE between rodent and humans in that TCE is metabolized more efficiently in rats and mice than humans, and a greater proportion of TCE is metabolized to DCA in rodents versus to TCA in humans. Studies that examined the induction of cardiac malformations with gestational exposures of rodents to various metabolites of TCE identified TCA and DCA as putative cardiac teratogens. Johnson et al. (1998a, b) and Smith et al. (1989) reported increased incidences of cardiac defects with gestational TCA exposures, while Smith et al. (1992) and Epstein et al. (1992) reported increased incidences following DCA exposures.

In all studies that observed increased cardiac defects, either TCE or its metabolites were administered during critical windows of *in utero* cardiac development, primarily during the entire duration of gestation, or during the period of major organogenesis (e.g., GD 6−15 in the rat). The study by Epstein et al. (1992) used dosing with DCA on discrete days of gestation and had identified gestation days 9 through 12 as a particularly sensitive period for eliciting high interventricular septal defects associated with exposures to TCE or its metabolites.

In the oral studies that identified increased incidences of cardiac malformations following gestational exposure to TCE, there was a broad range of administered doses at which effects were observed. In drinking water studies, Dawson et al. (1993) observed cardiac anomalies at 1.5 and 1,100 ppm (with no NOAEL) and Johnson et al. (2003, 2005) reported effects at 250 ppb (with a NOAEL of 2.5. ppb). One concern is the lack of a clear dose-response for the incidence of any specific cardiac anomaly or combination of anomalies was not identified, a disparity for which no reasonable explanation for this disparity has been put forth.

The analysis of the incidence data for cardiac defects observed in the Johnson et al. (2003, 2005) studies has been critiqued (Watson et al., 2006). Issues of concern that have been raised include the statistical analyses of findings on a per-fetus (rather than the more appropriate per-litter) basis (Benson, 2004), and the use of nonconcurrent control data in the analysis (Hardin et al., 2004). In response, the study author has further explained procedures used (Johnson, 2004) and has provided individual litter incidence data to the U.S. EPA for independent statistical analysis (P. Johnson, personal communication, 2008) (see Section 5.1.2.8, dose-response). In sum, while the studies by Dawson et al. (1993) and Johnson et al. (2003, 2005) have significant limitations, there is insufficient reason to dismiss their findings.

4.8.3.3.2.1. ***Mode of action for cardiac malformations*****.** A number of *in vitro* studies have been conducted to further characterize the potential for alterations in cardiac development that have been attributed to exposures with TCE and/or its metabolites. It was noted that many of the cardiac defects observed in humans and laboratory species (primarily rats and chickens) involved septal and valvular structures.

During early cardiac morphogenesis, outflow tract and atrioventricular (A-V) endothelial cells differentiate into mesenchymal cells. These mesenchymal cells have characteristics of smooth muscle-like myofibroblasts and form endocardial cushion tissue, which is the primordia of septa and valves in the adult heart. Events that take place in cardiac valve formation in mammals and birds are summarized by NRC (2006) and reproduced in Table 4-93.

Table 4-93. Events in cardiac valve formation in mammals and birds[a]

Stage and event	Structural description [b]
Early cardiac development	The heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothial layer. Extracellular matrix is between the two cell layers.
Epithelial-mesenchymal cell transformation	A subpopulation of endothelial sells lining the atrioventricular canal detaches from adjacent cells and invades the underlying extracellular matrix. Three events occur ➢ Endothelial cell activation (avian stage 14) ➢ Mesenchymal cell formation (avian stage 16) ➢ Mesenchymal cell migration into the extracellular matrix (avian stages 17 and 18).
Mesenchymal cell migration and proliferation	Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and proliferate to populate the atrioventricular (A-V) canal extracellular matrix.
Development of septa and valvular structures	Cardiac mesenchyme provides cellular constituents for ➢ Septum intermedium ➢ Valvular leaflets of the mitral and tricuspid A-V valves. The septum intermedium subsequently contributes to ➢ Lower portion of the interatrial septum ➢ Membranous portion of the interventricular septum.

[a]As summarized in NRC (2006)
[b]Markwald et al. (1984, 1996), Boyer et al. (2000).

Methods have been developed to extract the chick stage 16 atrioventricular canal from the embryo and culture it on a hydrated collagen gel for 24−48 hours, allowing evaluation of the described stages of cardiac development and their response to chemical treatment. Factors that have been shown to influence the induction of endocardial cushion tissue include molecular components such as fibronectin, laminin, and galactosyltransferase (Mjaatvedt et al., 1987; Loeber and Runyan, 1990), components of the extracellular matrix (Mjaatvedt et al., 1991), and smooth muscle α-actin and transforming growth factor β3 (Nakajima et al., 1997; Ramsdell and Markwald, 1997).

Boyer et al. (2000) utilized the *in vitro* chick A-V canal culture system to examine the molecular mechanism of TCE effects on cardiac morphogenesis. A-V canal explants from stage 16 chick embryos (15/treatment level) were placed onto collagen gels and treated with 0, 50,

100, 150, 200, or 250-ppm TCE and incubated for a total of 54 hours. Epithelial-mesenchymal transformation, endothelial cell density, cell migration, and immunohistochemistry were evaluated. TCE treatment was found to inhibit endothelial cell activation and normal mesenchymal cell transformation, endothelial cell-cell separation, and protein marker expression (i.e., transcription factor Mox-1 and extracellular matrix protein fibrillin 2). Mesenchymal cell migration was not affected, nor was the expression of smooth muscle α-actin. The study authors proposed that TCE may cause cardiac valvular and septal malformations by inhibiting endothelial separation and early events of mesenchymal cell formation. Hoffman et al. (2004) has proposed alternatively that TCE may be affecting the adhesive properties of the endocardial cells. No experimental data are currently available that address the levels of TCE in cardiac tissue *in vivo*, resulting in some questions (Dugard, 2000) regarding the relevance of these mechanistic findings to human health risk assessment.

In a study by Mishima et al. (2006), White Leghorn chick whole embryo cultures (stage 13 and 14) were used to assess the susceptibility of endocardial epithelial-mesenchymal transformation in the early chick heart to TCE at analytically determined concentrations of 0, 10, 20, 40, or 80 ppm. This methodology maintained the anatomical relationships of developing tissues and organs, while exposing precisely staged embryos to quantifiable levels of TCE and facilitating direct monitoring of developmental morphology. Following 24 hours of incubation the numbers of mesenchymal cells in the inferior and superior AV cushions were counted. TCE treatment significantly reduced the number of mesenchymal cells in both the superior and inferior AV cushions at 80 ppm.

Ou et al. (2003) examined the possible role of endothelial nitric oxide synthase (which generates nitric oxide that has an important role in normal endothelial cell proliferation and hence normal blood vessel growth and development) in TCE-mediated toxicity. Cultured proliferating bovine coronary endothelial cells were treated with TCE at 0−100 μM and stimulated with a calcium ionophore to determined changes in endothelial cells and the generation of endothelial nitric oxide synthase, nitric oxide, and superoxide anion. TCE was shown to alter heat shock protein interactions with endothelial nitric oxide synthase and induce endothelial nitric oxide synthase to shift nitric oxide to superoxide-anion generation. These findings provide insight into how TCE impairs endothelial proliferation.

Several studies have also identified a TCE-related perturbation of several proteins involved in regulation of intracellular Ca^{2+}. After 12 days of maternal exposure to TCE in drinking water, *Serca2a* (sarcoendoplasmic reticulum Ca^{2+} ATPase) mRNA expression was reduced in rat embryo cardiac tissues (Collier et al., 2003). Selmin et al. (2008) conducted a microarray analysis of a P19 mouse stem cell line exposed to 1-ppm TCE *in vitro*, identifying altered expression of *Ryr* (ryanodine receptor isoform 2). Caldwell et al. (2008) used real-time

PCR and digital imaging microscopy to characterize the effects of various doses of TCE on gene expression and Ca^{2+} response to vasopressin in rat cardiac myocytes (H9c2) *Serca2a* and *Ryr2* expression were reduced at 12 and 48 hours following exposure to TCE. Additionally, Ca^{2+} response to vasopressin was altered following TCE treatment. Overall, these data suggest that TCE may disrupt the ability to regulate cellular Ca^{2+} fluxes, leading to morphogenic consequences in the developing heart. This remains an open area of research.

Thus, in summary, a number of studies have been conducted in an attempt to characterize the MOA for TCE-induced cardiac defects. A major research focus has been on disruptions in cardiac valve formation, using avian *in ovo* and *in vitro* studies. These studies demonstrated treatment-related alterations in endothelial cushion development that could plausibly be associated with defects involving septal and valvular morphogenesis in rodents and chickens. However, a broad array of cardiac malformations has been observed in animal models following TCE exposures (Dawson et al., 1993; Johnson et al., 2003, 2005), and other evidence of molecular disruption of Ca^{2+} during cardiac development has been examined (Caldwell et al., 2008; Collier et al., 2003; Selmin et al., 2008) suggesting the possible existence of multiple MOAs.

4.8.3.3.2.2. ***Association of peroxisome proliferator activated receptor alpha (PPAR) with developmental outcomes*.** The PPARs are ligand activated receptors that belong to the nuclear hormone receptor family. Three isotypes have been identified (PPARα, PPARδ [also known as PPARβ], and PPARγ). These receptors, upon binding to an activator, stimulate the expression of target genes implicated in important metabolic pathways. In rodents, all three isotypes show specific time and tissue-dependent patterns of expression during fetal development and in adult animals. In development, they have been especially implicated in several aspects of tissue differentiation, e.g., of the adipose tissue, brain, placenta and skin. Epidermal differentiation has been linked strongly with PPARα and PPARδ (Michalik et al., 2002). PPARα starts late in development, with increasing levels in organs such as liver, kidney, intestine, and pancreas; it is also transiently expressed in fetal epidermis and CNS (Braissant and Wahli, 1998) and has been linked to phthalate-induced developmental and testicular toxicity (Corton and Lapinskas, 2005). Liver, kidney, and heart are the sites of highest PPARα expression (Toth et al., 2007). PPARδ and PPARγ have been linked to placental development and function, with PPARγ found to be crucial for vascularization of the chorioallantoic placenta in rodents (Wendling et al., 1999), and placental anomalies mediated by PPARγ have been linked to rodent cardiac defects (Barak et al., 2008). While it might be hypothesized that there is some correlation between PPAR signaling, fetal deaths, and/or cardiac defects observed following TCE exposures in rodents, no definitive data have been generated that elucidate a possible PPAR-mediated MOA for these outcomes.

4.8.3.3.2.3. ***Summary of the weight of evidence on cardiac malformations.*** The evidence for an association between TCE exposures in the human population and the occurrence of congenital cardiac defects is not particularly strong. Many of the epidemiological study designs were not sufficiently robust to detect exposure-related birth defects with a high degree of confidence. However, two well-conducted studies by ATSDR (2006, 2008) clearly demonstrated an elevation in cardiac defects. It could be surmised that the identified cardiac defects were detected because they were severe, and that additional cases with less severe cardiac anomalies may have gone undetected.

The animal data provide strong, but not unequivocal, evidence of the potential for TCE-induced cardiac malformations following oral exposures during gestation. Strengths of the evidence are the duplication of the adverse response in several studies from the same laboratory group, detection of treatment-related cardiac defects in both mammalian and avian species (i.e., rat and chicken), general cross-study consistency in the positive association of increased cardiac malformations with test species (i.e., rat), route of administration (i.e., oral), and the methodologies used in cardiac morphological evaluation (i.e., fresh dissection of fetal hearts). Furthermore, when differences in response are observed across studies they can generally be attributed to obvious methodological differences, and a number of *in ovo* and *in vitro* studies demonstrate a consistent and biologically plausible MOA for one type of malformation observed. Weaknesses in the evidence include lack of a clear dose-related response in the incidence of cardiac defects, and the broad variety of cardiac defects observed, such that they cannot all be grouped easily by type or etiology.

Taken together, the epidemiological and animal study evidence raise sufficient concern regarding the potential for developmental toxicity (increased incidence of cardiac defects) with *in utero* TCE exposures.

4.8.3.3.3. ***Other structural developmental outcomes.*** A summary of other structural developmental outcomes that have been associated with TCE exposures is presented in Table 4-94.

In humans, a variety of birth defects other than cardiac have been observed. These include total birth defects (Bove, 1996; Bove et al., 1995; AZ DHS, 1988; ATSDR, 2001), CNS birth defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), eye/ear birth anomalies (Lagakos et al., 1986); oral cleft defects (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986; Lorente et al., 2000); kidney/urinary tract disorders (Lagakos et al., 1986); musculoskeletal birth anomalies (Lagakos et al., 1986); anemia/blood disorders (Burg and Gist, 1999); and lung/respiratory tract disorders (Lagakos et al., 1986). While some of these results were statistically significant, they have not been reported elsewhere. Occupational cohort

studies, while not reporting positive results, are generally limited by the small number of observed or expected cases of birth defects (Lorente et al., 2000; Tola et al., 1980; Taskinen et al., 1989).

Table 4-94. Summary of other structural developmental outcomes associated with TCE exposures

Finding	Species	Citations
Eye/ear birth anomalies	Human	Lagakos et al., 1986
	Rat	Narotsky, 1995 Narotsky and Kavlock, 1995
Oral cleft defects	Human	Bove, 1996 Bove et al., 1995 Lagakos et al., 1986 Lorente et al., 2000
Kidney/urinary tract disorders	Human	Lagakos et al., 1986
Musculoskeletal birth anomalies	Human	Lagakos et al., 1986
Anemia/blood disorders	Human	Burg and Gist, 1999
Lung/respiratory tract disorders	Human	Lagakos et al., 1986
	Mouse	Das and Scott, 1994
Skeletal	Rat	Healy et al., 1982
Other*	Human	ATSDR, 2001

*As reported by the authors.

In experimental animals, a statistically significant increase in the incidence of fetal eye defects, primarily micropththalmia and anopththalmia, manifested as reduced or absent eye bulge, was observed in rats following gavage administration of 1,125 mg/kg/d TCE during the period of organogenesis (Narotsky et al., 1995; Narotsky and Kavlock, 1995). Dose-related nonsignificant increases in the incidence of Fischer 344 rat pups with eye defects were also observed at lower dose levels (101, 320, 475, 633, and 844 mg/kg/d) in the Narotsky et al. (1995) study (also reported in Barton and Das [1996]). However, no other developmental or reproductive toxicity studies identified abnormalities of eye development following TCE exposures. For example, in a study reported by Warren et al. (2006), extensive computerized morphometric ocular evaluation was conducted in Sprague-Dawley rat fetuses that had been examined for cardiac defects by Fisher et al. (2001); the dams had been administered TCE

(500 mg/kg/d), DCA (300 mg/kg/d), or TCA (300 mg/kg/d) during gestation days 6−15. No ocular defects were found with TCE exposures; however, significant reductions in the lens area, globe area, and interocular distance were observed with DCA exposures, and nonsignificant decreases in these measures as well as the medial canthus distance were noted with TCA exposures. Developmental toxicity studies conducted by Smith et al. (1989, 1992) also identified orbital defects (combined soft tissue and skeletal abnormalities) in Long Evans rat fetuses following GD 6−15 exposures with TCA and DCA (statistically or biologically significant at ≥800 mg/kg/d and ≥900 mg/kg/d, respectively). Overall, the study evidence indicates that TCE and its oxidative metabolites can disrupt ocular development in rats. In addition to the evidence of alteration to the normal development of ocular structure, these findings may also be an indicator of disruptions to nervous system development. It has been suggested by Warren et al. (2006) and Williams and DeSesso (2008) that the effects of concern (defined as statistically significant outcomes) are observed only at high dose levels and are not relevant to risk assessment for environmental exposures. On the other hand, Barton and Das (1996) point out that benchmark dose modeling of the quantal eye defect incidence data provides a reasonable approach to the development of oral toxicity values for TCE human health risk assessment. It is also noted that concerns may exist not only for risks related to low level environmental exposures, but also for risks resulting from acute or short-term occupational or accidental exposures, which may be associated with much higher inadvertent doses.

It was also notable that a study using a single intraperitoneal dose of 3,000 mg/kg TCE to mice during late gestation (GD 17) identified apparent delays in lung development and increased neonatal mortality (Das and Scott, 1994). No further evaluation of this outcome has been identified in the literature.

Healy et al. (1982) did not identify any treatment-related fetal malformations following inhalation exposure of pregnant inbred Wistar rats to 0 or 100 ppm (535 mg/m^3) on GD 8−21. In this study, significant differences between control and treated litters were observed as an increased incidence of minor ossification variations (p = 0.003) (absent or bipartite centers of ossification).

4.8.3.3.4. ***Developmental neurotoxicity.*** Studies that address effects of TCE on the developing nervous system are discussed in detail in Section 4.3, addressed above in the sections on human developmental toxicity (Section 4.8.3) and on mammalian studies (Section 4.8.3.2.1) by route of exposure, and summarized in Table 4-95. The available data collectively suggest that the developing brain is susceptible to TCE exposures.

Table 4-95. Summary of developmental neurotoxicity associated with TCE exposures

Positive findings	Species	Citations
CNS defects, neural tube defects	Human	ATSDR, 2001
		Bove, 1996; Bove et al., 1995
		Lagakos et al., 1986
Eye defects	Rat	Narotsky, 1995; Narotsky and Kavlock, 1995
Delayed newborn reflexes	Human	Beppu, 1968
Impaired learning or memory	Human	Bernad et al., 1987, abstract
		White et al., 1997
Aggressive behavior	Human	Bernad et al., 1987, abstract
	Rat	Blossom et al., 2008
Hearing impairment	Human	ATSDR, 2003a; Burg et al., 1995; Burg and Gist, 1999
		Beppu, 1968
Speech impairment	Human	ATSDR, 2003a; Burg et al., 1995; Burg and Gist, 1999
		White et al., 1997
Encephalopathy	Human	White et al., 1997
Impaired executive function	Human	White et al., 1997
Impaired motor function	Human	White et al., 1997
Attention deficit	Human	Bernad et al., 1987, abstract
ASD	Human	Windham et al., 2006
Delayed or altered biomarkers of CNS development	Rat	Isaacson and Taylor, 1989 Noland-Gerbec et al., 1986 Westergren et al., 1984
Behavioral alterations	Mice	Blossom et al., 2008 Fredriksson et al., 1993
	Rat	George et al., 1986 Taylor et al., 1985

In humans, CNS birth defects were observed in a few studies (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). Postnatally, observed adverse effects in humans include delayed newborn reflexes following use of TCE during childbirth (Beppu, 1968), impaired learning or memory (Bernad et al., 1987, abstract; White et al., 1997); aggressive

behavior (Bernad et al., 1987, abstract); hearing impairment (Beppu, 1968; Burg et al., 1995; Burg and Gist, 1999; ATSDR, 2003a); speech impairment (Berg et al., 1995; Burg and Gist, 1999; White et al., 1997); encephalopathy (White et al., 1997); impaired executive and motor function (White et al., 1997); attention deficit (Bernad et al., 1987, abstract; White et al., 1997), and autism spectrum disorder (Windham et al., 2006). While there are broad developmental neurotoxic effects that have been associated with TCE exposure, there are many limitations in the studies.

More compelling evidence for the adverse effect of TCE exposure on the developing nervous system is found in the animal study data, although a rigorous evaluation of potential outcomes has not been conducted. For example, there has not been an assessment of cognitive function (i.e., learning and memory) following developmental exposures to TCE, nor have most of the available studies characterized the pre- or postnatal exposure of the offspring to TCE or its metabolites. Nevertheless, there is evidence of treatment-related alterations in brain development and in behavioral parameters (e.g., spontaneous motor activity and social behaviors) associated with exposures during neurological development. The animal study database includes the following information: Following inhalation exposures of 150 ppm to mice during mating and gestation, the specific gravity of offspring brains were significantly decreased at postnatal time points through the age of weaning; however, this effect did not persist to 1 month of age (Westergren et al., 1984). In studies reported by Taylor et al. (1985), Isaacson and Taylor (1989), and Noland-Gerbec et al. (1986), 312 mg/L exposures in drinking water that were initiated 2 weeks prior to mating and continued to the end of lactation resulted, respectively, in (a) significant increases in exploratory behavior at postnatal days 60 and 90, (b) reductions in myelination in the brains of offspring at weaning, and (c) significantly decreased uptake of 2-deoxyglucose in the neonatal rat brain (suggesting decreased neuronal activity). Ocular malformations in rats observed by Narotsky (1995) and Narotsky and Kavlock (1995) following maternal gavage doses of 1,125 mg/kg/d during gestation may also be indicative of alterations of nervous system development. Gestational exposures to mice (Fredriksson et al., 1993) resulted in significantly decreased rearing activity on postnatal Day 60, and dietary exposures during the course of a continuous breeding study in rats (George et al., 1986) found a significant trend toward increased time to cross the first grid in open field testing. In a study by Blossom et al. (2008), alterations in social behaviors (deficits in nest-building quality and increased aggression in males) were observed in pubertal-age MRL +/+ mice that had been exposed to 0.1 mg/mL TCE via drinking water during prenatal and postnatal development (until PND 42). Dorfmueller et al. (1979) was the only study that assessed neurobehavioral endpoints following *in utero* exposure (maternal inhalation exposures of 1,800 ± 200 ppm during gestation) and found no adverse effects that could be attributed to TCE exposure. Specifically, an

automated assessment of ambulatory response in a novel environment on postnatal days 10, 20 and 100, did not identify any effect on general motor activity of offspring.

4.8.3.3.5. ***Developmental immunotoxicity.*** Studies that address the developmental immunotoxic effects of TCE are discussed in detail in Section 4.6, addressed above in the sections on human developmental toxicity (Section 4.8.3) and on mammalian studies (Section 4.8.3.2.1) by route of exposure, and summarized in Table 4-96.

Table 4-96. Summary of developmental immunotoxicity associated with TCE exposures

Finding	Species (strain)	Citations
Significant reduction in Th1 IL-2 producing cells	Human	Lehmann et al., 2002
Altered immune response	Human	Byers et al., 1988
Suppression of PFC responses, increased T-cell subpopulations, decreased spleen cellularity, and increased hypersensitivity response	Mouse (B6C3F1)	Peden-Adams et al., 2006
Altered splenic and thymic T-cell subpopulations	Mouse (MRL +/+)	Peden-Adams et al., 2008
Altered thymic T-cell subpopulations; transient increased proinflammatory cytokine production by T-cells; increased autoantibody levels and IgG	Mouse (MRL +/+)	Blossom and Doss, 2007
Increased proinflammatory cytokine production by T-cells	Mouse (MRL +/+)	Blossom et al., 2008

Two epidemiological studies that addressed potential immunological perturbations in children that were exposed to TCE were reported by Lehmann et al. (2001, 2002). In the 2001 study, no association was observed between TCE and allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells, in premature neonates and 36-month-old neonates that were at risk of atopy. In the 2002 study, there was a significant reduction in Th1 IL-2 producing cells. Another study observed altered immune response in family members of those diagnosed with childhood leukemia, including 13 siblings under age 19 at the time of exposure, but an analysis looking at only these children was not done (Byers et al., 1988).

Several studies were identified (Peden-Adams et al., 2006, 2008; Blossom and Doss, 2007; Blossom et al., 2008) which assessed the potential for developmental immunotoxicity in mice following oral (drinking water) TCE exposures during critical pre- and postnatal stages of immune system development. Peden-Adams et al. (2006) noted evidence of immune system

perturbation (suppression of PFC responses, increased T-cell subpopulations, decreased spleen cellularity, and increased hypersensitivity response) in B6C3F1 offspring following *in utero* and 8 weeks of postnatal exposures to TCE. Evidence of autoimmune response was not observed in the offspring of this nonautoimmune-prone strain of mice. However, in a study by Peden-Adams et al. (2008) MRL +/+ mice, which are autoimmune-prone, were exposed from conception until 12 months of age. Consistent with the Peden-Adams et al. (2006) study, no evidence of increased autoantibody levels was observed in the offspring. In two other studies focused on autoimmune responses following drinking water exposures of MRL +/+ mice to TCE during *in utero* development and continuing until the time of sexual maturation, Blossom and Doss (2007) and Blossom et al. (2008) reported some peripheral blood changes that were indicative of treatment-related autoimmune responses in offspring. Positive response levels were 0.5 and 2.5 mg/mL for Blossom and Doss (2007) and 0.1 mg/mL for Blossom et al. (2008). None of these studies were designed to extensively evaluate recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures. Consistency in response in these animal studies was difficult to ascertain due to the variations in study design (e.g., animal strain used, duration of exposure, treatment levels evaluated, timing of assessments, and endpoints evaluated). Likewise, the endpoints assessed in the few epidemiological studies that evaluated immunological outcomes following developmental exposures to TCE were dissimilar from those evaluated in the animal models, and so provided no clear cross-species correlation. The most sensitive immune system response noted in the studies that exposed developing animals were the decreased PFC and increased hypersensitivity observed by Peden-Adams et al. (2006); treatment-related outcomes were noted in mice exposed in the drinking water at a concentration of 1,400 ppb. None of the other studies that treated mice during immune system development assessed these same endpoints; therefore, direct confirmation of these findings across studies was not possible. It is noted, however, that similar responses were not observed in studies in which adult animals were administered TCE (e.g., Woolhiser et al., 2006), suggesting increased susceptibility in the young. Differential lifestage-related responses have been observed with other diverse chemicals (e.g., diethylstilbestrol; diazepam; lead; 2,3,7,8-tetrachlorobenzo-*p* dioxin; and tributyltin oxide) in which immune system perturbations were observed at lower doses and/or with greater persistence when tested in developing animals as compared to adults (Luebke et al., 2006). Thus, such an adverse response with TCE exposure is considered biologically plausible and an issue of concern for human health risk assessment.

4.8.3.3.6. ***Childhood cancers.*** A summary of childhood cancers that have been associated with TCE exposures discussed above is presented in Table 4-97. A summary of studies that observed

childhood leukemia is also discussed in detail in Section 4.6.1.3 and Section 4.8.3.1.2.4 contains details of epidemiologic studies on childhood brain cancer.

Table 4-97. Summary of childhood cancers associated with TCE exposures

Finding	Species	Citations
Leukemia	Human	AZ DHS, 1988, 1990a
		AZ DHS, 1990c
		Cohn et al., 1994
		Cutler et al., 1986; Costas et al., 2002; Lagakos et al., 1986; MA DPH, 1997
		Lowengart et al., 1987
		McKinney et al., 1991
		Shu et al., 1999
Neuroblastoma	Human	De Roos et al., 2001
		Peters et al., 1981, 1985

A nonsignificant increased risk of leukemia diagnosed during childhood has been observed in a number of studies examining TCE exposure (AZ DHS, 1998, 1990a, c; Cohn et al., 1994; Costas et al., 2002; Lagakos et al., 1986; Lowengart et al., 1987; MA DPH, 1997; McKinney et al., 1991; Shu et al., 1999). However, other studies did not observed an increased risk for childhood leukemia after TCE exposure (AZ DHS, 1990b, 1997; Morgan and Cassady, 2002), possibly due to the limited number of cases or the analysis based on multiple solvents. CNS cancers during childhood have been reported on in a few studies. Neuroblastomas were not statistically elevated in one study observing parental exposure to multiple chemicals, including TCE (De Roos et al., 2001). Brain tumors were observed in another study, but the odds ratio could not be determined (Peters et al., 1981, 1985). CNS cancers were not elevated in other studies (AZ DHS, 1990c; Morgan and Cassady, 2002). Other studies did not see an excess risk of total childhood cancers (ATSDR, 2006; Morgan and Cassady, 2002).

A follow-up study of the Camp Lejeune cohort that will examine childhood cancers (along with birth defects) was initiated in 1999 (ATSDR, 2003b), is expected to be completed soon (GAO, 2007a, b; ATSDR, 2009), and may provide additional insight.

No studies of cancers in experimental animals in early lifestages have been identified.

4.9. OTHER SITE-SPECIFIC CANCERS

4.9.1. Esophageal Cancer

Increasing esophageal cancer incidence has been observed in males, but not females in the United States between 1975 and 2002, a result of increasing incidence of esophageal adenocarcinoma (Ward et al., 2006). Males also have higher age-adjusted incidence and mortality rates (incidence, 7.8 per 100,000; mortality, 7.8 per 100,000) than females (incidence, 2.0 per 100,000; mortality, 1.7 per 100,000) (Ries et al., 2008). Survival for esophageal cancer remains poor and age-adjusted mortality rates are just slightly lower than incidence rates. Major risk factors associated with esophageal cancer are smoking and alcohol for squamous cell carcinoma, typically found in the upper third of the esophagus, and obesity, gastroesophageal reflux, and Barrett's esophagus for adenocarcinoma that generally occurs in the lower esophagus (Ward et al., 2006).

Seventeen epidemiologic studies on TCE exposure reported relative risks for esophageal cancer (Garabrant et al., 1988; Blair et al., 1989; Costa et al., 1989; Siemiatycki, 1991; Greenland et al., 1994; Blair et al., 1998; Boice et al., 1999, 2006; Ritz, 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; ATSDR, 2004, 2006; Zhao et al., 2005; Sung et al., 2007; Clapp and Hoffman, 2008; Radican et al., 2008). Ten studies had high likelihood of TCE exposure in individual study subjects and were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis (Siemiatycki, 1991; Greenland et al., 1994; Blair et al., 1998; Boice et al., 1999, 2006; Ritz, 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Radican et al., 2008). Four studies with high quality information (Axelson et al., 1994; Anttila et al., 1995; Blair et al., 1998 [Incidence]; Morgan et al., 1998) do not present relative risk estimates for esophageal cancer and TCE exposure nor do two other studies which carry less weight in the analysis because of design limitations (Sinks et al., 1992; Henschler et al., 1995). Only Raaschou-Nielsen et al. (2003) examines esophageal cancer histologic type, an important consideration given differences between suspected risk factors for adenocarcinoma and those for squamous cell carcinoma. Appendix B identifies these study's design and exposure assessment characteristics.

Several population case-control studies (Yu et al., 1988; Gustavsson et al., 1998; Parent et al., 2000; Weiderpass et al., 2003; Engel et al., 2002; Ramanakumar et al., 2008; Santibañez et al., 2008) examine esophageal cancer and organic solvents or occupational job titles with past TCE use documented (Bakke et al., 2006). Relative risk estimates in case-control studies that examine metal occupations or job titles, or solvent exposures are found in Table 4-98. The lack of exposure assessment to TCE, low prevalence of exposure to chlorinated hydrocarbon solvents, or few exposed cases and controls in those studies lowers their sensitivity for informing evaluations of TCE and esophageal cancer.

Table 4-98. Selected observations from case-control studies of TCE exposure and esophageal cancer

Study population	Exposure group	All esophageal cancers		Squamous cell cancer		Adenocarcinoma		Reference
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	
Population of regions in Eastern Spain								Santibañez et al., 2008
	Metal molders, welders, etc.	0.94 (0.14, 6.16)	3	0.40 (0.05, 3.18)	2	3.55 (0.28, 44.70)	1	
	Metal–processing plant operators	1.14 (0.29, 4.44)	5	1.23 (0.23, 6.51)	4	0.86 (0.08, 8.63)	1	
	Chlorinated hydrocarbon solvents							
	Low exposure	1.05 (0.15, 7.17)	2		0	4.92 (0.69, 34.66)	2	
	High exposure	1.76 (0.40, 7.74)	6	2.18 (0.41, 11.57)	5	3.03 (0.28, 32.15)	1	
Population of Montreal, Canada								Ramanakumar et al., 2008; Parent et al., 2000
	Painter, Metal coatings							
	Any exposure	1.3 (0.4, 4.2)	6					
	Substantial exposure	4.2 (1.1, 17.0)	4					
	Solvents							
	Any exposure	1.1 (0.7, 1.7)	39	1.4 (0.8, 2.5)	30			
	Nonsubstantial exposure	1.0 (0.5, 1.9)	16	1.3 (0.6, 2.6)	12			
	Substantial exposure	1.1 (0.6, 1.9)	39	1.4 (0.8, 2.5)	30			
Population of Sweden								Janssen et al., 2006a, b
	Organic solvents							
	No exposure			1.0	145	1.0	128	
	Moderate exposure			0.7 (0.4, 1.5)	15	1.2 (0.6, 2.3)	14	
	High exposure			1.3 (0.7, 2.3)	21	1.4 (0.7, 2.5)	18	
	Test for trend			$p = 0.47$		$p = 0.59$		
	No exposure			1.0		1.0		
	Moderate exposure			0.5 (0.1, 3.9)*	1	0.4 (0.1, 1.5)*	2	
	High exposure			0.4 (0.1, 1.8)*	2	0.9 (0.5, 1.6)*	12	
	Test for trend			$p = 0.44$		$p = 0.36$		

Table 4-98. Selected observations from case-control studies of TCE exposure and esophageal cancer (continued)

Study population	Exposure group	All esophageal cancers		Squamous cell cancer		Adenocarcinoma		Reference
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	
Population of Finland (Females)								Weiderpass et al., 2003
	Chlorinated hydrocarbon solvents							
	Low level exposure	0.95 (0.54, 1.66)	Not reported					
	High level exposure	0.62 (0.34, 1.13)	Not reported					
Population of NJ, CT, WA State								Engel et al., 2002
	Precision metal workers	Not reported		0.7 (0.3, 1.5)	12	1.4 (0.8, 2.3)	25	
	Metal product manufacturing	Not reported		0.8 (0.3, 1.8)	15	1.3 (0.8, 2.3)	26	

*Jansson et al. (2006b) is a registry-based study of the Swedish Construction Worker Cohort. Relative risks are incidence rate ratios from Cox regression analysis using calendar time and adjustment for attained age, calendar period at entry into the cohort, tobacco smoking status at entry into the cohort and BMI at entry into the cohort.

Table 4-99 presents risk estimates for TCE exposure and esophageal cancer observed in cohort, PMR, case-control, and geographic based studies. Ten studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on job-exposure matrices or biomarker monitoring) reported risk estimates for esophageal cancer (Siemtiatycki, 1991; Greenland et al., 1994; Blair et al., 1998; Boice et al., 1999; Ritz et al., 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Boice et al., 2006; Radican et al., 2008). Some evidence for association with esophageal cancer and overall TCE exposure comes from studies with high likelihood of TCE exposure (5.6, 95% CI: 0.7, 44.5 [Blair et al., 1998] and 1.88, 95% CI: 0.61, 5.79 [Radican et al., 2008, which was an update of Blair et al., 1998 with an additional 10 years of follow-up]; 4.2, 95% CI: 1.5, 9.2, [Hansen et al., 2001]; 1.2, 95% CI: 0.84, 1.57 [Raaschou-Nielsen et al., 2003]). Two studies support an association with adenocarcinoma histologic type of esophageal cancer and TCE exposure (five of the six observed esophageal cancers were adenocarcinomas [less than 1 expected; Hansen et al., 2001]); 1.8, 95% CI: 1.2, 2.7 (Raaschou-Nielsen et al., 2003). Risk estimates in other high-quality studies are based on few deaths, low statistical power to detect a doubling of esophageal cancer risk, and confidence intervals which include a risk estimate of 1.0 (no increased risk).

Seven other studies (Garabrant et al., 1988; Blair et al., 1989; Costa et al., 1989; Sung et al., 2007; ATSDR, 2004, 2006; Clapp and Hoffman, 2008) with lower likelihood for TCE exposure, in addition to limited statistical power and other design limitations, observed relative risk estimates between 0.21 (95% CI: 0.0.01, 1.17) (Costa et al., 1989) to 1.14 (95% CI: 0.62, 1.92) (Garabrant et al., 1988). For these reasons, esophageal cancer observations in these studies are not inconsistent with Blair et al. (1998) and its update Radican et al. (2008), Hansen et al., (2001), or Raaschou-Nielsen et al. (2003). No study reported a statistically significant deficit in the esophageal cancer risk estimate and overall of TCE exposure. Of those studies with exposure-response analyses, a pattern of increasing esophageal cancer relative risk with increasing exposure metric is not generally noted (Siemiatycki, 1991; Blair et al., 1998; Boice et al., 1999; Zhao et al., 2005; Radican et al., 2008) except for Hansen et al. (2001) and Raaschou-Nielsen et al. (2003). In these last two studies, esophageal cancer relative risk estimates associated with long employment duration were slightly higher (SIR: 6.6, 95% CI: 1.8, 7.0.8, 3.7 [Hansen et al., 2001]; SIR: 1.9, 95% CO: 0.8, 3.7 [Raaschou-Nielsen et al., 2003]) than those for short employment duration (SIR: 4.4, 95% CI: 0.5, 19 [Hansen et al., 2001]; SIR: 1.7, 95% CI: 0.6, 3.6 [Raaschou-Nielsen et al., 2003]). Hansen et al. (2001) also reports risk for two other TCE exposure surrogates, average intensity and cumulative exposure, and in both cases observed lower risk estimates with the higher exposure surrogate.

Table 4-99. Summary of human studies on TCE exposure and esophageal cancer

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Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al., 2005
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00[a]	9	
	Med cumulative TCE score	1.66 (0.62, 4.41)[b]	8	
	High TCE score	0.82 (0.17, 3.95)[b]	2	
	p for trend	$p = 0.974$		
All employees at electronics factory (Taiwan)				Sung et al., 2007
	Males	Not reported		
	Females	1.16 (0.0.14, 4.20)[c]	2	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure, all subjects	1.2 (0.84, 1.57)	44	
	Any exposure, males	1.1 (0.81, 1.53)	40	
	Any exposure, females	2.0 (0.54, 5.16)	4	
	Any exposure, males	1.8 (1.15, 2.73)[d]	23	
	Any exposure, females		0 (0.4 exp)[d]	
	Exposure lag time			
	20 yrs	1.7 (0.8, 3.0)[d]	10	
	Employment duration			
	<1 yr	1.7 (0.6, 3.6)[d]	6	
	1−4.9 yrs	1.9 (0.9, 3.6)[d]	9	
	≥5 yrs	1.9 (0.8, 3.7)[d]	8	
	Subcohort with higher exposure			
	Any TCE exposure	1.7 (0.9, 2.9)[d]	13	
	Employment duration			
	1−4.9 yrs	1.6 (0.6, 3.4)[d]	6	
	≥5 yrs	1.9 (0.8, 3.8)[d]	7	
Biologically-monitored Danish workers		4.0 1.5, 8.72)	6	Hansen et al., 2001
	Any TCE exposure, males	4.2 (1.5, 9.2)	6	
	Adenocarcinoma histologic type	3.6 (1.2, 8.3)[e]	5	
	Any TCE exposure, females		0 (0.1 exp)	
	Cumulative exposure (Ikeda)			
	<17 ppm-yr	6.5 (1.3, 19)	3	
	≥17 ppm-yr	4.2 (1.5, 9.2)	3	
	Mean concentration (Ikeda)			
	<4 ppm	8.0 (2.6, 19)	5	
	4+ ppm	1.3 (0.02, 7.0)	1	

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Table 4-99. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Employment duration			
	<6.25 yr	4.4 (0.5, 16)	2	
	≥6.25 yr	6.6 (1.8, 17)	4	
Aircraft maintenance workers from Hill Air Force Base				Blair et al., 1998
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	Not reported		
	5−25 ppm-yr	Not reported		
	>25 ppm-yr	Not reported		
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	Not reported		
	5−25 ppm-yr	Not reported		
	>25 ppm-yr	Not reported		
Biologically-monitored Finnish workers				Anttila et al., 1995
	All subjects	Not reported		
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	Not reported		
	6+ ppm	Not reported		
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	Exposed workers	Not reported		
Biologically-monitored Swedish workers				Axelson et al., 1994
	Any TCE exposure, males	Not reported		
	Any TCE exposure, females	Not reported		
Cardboard manufacturing workers, Atlanta area, GA				Sinks et al., 1992
	All subjects	Not reported		
Cohort and PMR studies-mortality				
Computer manufacturing workers (IBM), NY				Clapp and Hoffman, 2008
	Males	1.12 (0.30, 2.86)[f]		
		5.24 (0.13, 29.2)[f]		

Table 4-99. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	0.88 (0.18, 2.58)	3	Boice et al., 2006
	Any exposure to TCE	Not reported		Zhao et al., 2005
	Low cumulative TCE score	1.00[a]	18	
	Medium cumulative TCE score	1.40 (0.70, 2.82)[b]	15	
	High TCE score	1.27 (0.52, 3.13)[b]	7	
	p for trend	$p = 0.535$		
View-Master employees				ATSDR, 2004
	Males	0.62 (0.02, 3.45)[f]	1	
	Females		0 (1.45 exp)[f]	
All employees at electronics factory (Taiwan)				Chang et al., 2003
	Males		0 (3.34 exp)	
	Females		0 (0.83 exp)	
United States uranium-processing workers (Fernald)				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration	2.61 (0.99, 6.88)[g]	12	
	Moderate TCE exposure, >2 yrs duration		0	
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure	0.83 (0.34, 1.72)	7	
	Routine-intermittent[a]	Not presented	11	
	Duration of exposure			
	0 yrs	1.0[a]	28	
	<1 yr	0.23 (0.05, 0.99)	2	
	1−4 yrs	0.57 (0.20, 1.67)	4	
	≥5 yrs	0.91 (0.38, 2.22)	7	
	p for trend	$p > 0.20$		
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	Not reported		
	Low intensity (<50 ppm)			
	High intensity (>50 ppm)			
	TCE subcohort (Cox Analysis)	Not reported		
	Never exposed			
	Ever exposed			
	Peak	Not reported		
	No/Low			
	Medium/high			

Table 4-99. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Cumulative	Not reported		
	Referent			
	Low			
	High			
Aircraft maintenance workers (Hill AFB, UT)				Blair et al., 1998
	TCE subcohort	5.6 (0.7, 44.5) [a]	10	
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	Not reported[h]	3	
	5−25 ppm-yr	Not reported[h]	2	
	>25 ppm-yr	Not reported[h]	4	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	3.6 (0.2, 58)	1	
	5−25 ppm-yr		0	
	>25 ppm-yr		0	
	TCE subcohort	1.88 (0.61, 5.79)	17	Radican et al., 2008
	Males, cumulative exposure	1.66 (0.48, 5.74)	15	
	0	1.0[a]		
	<5 ppm-yr	1.84 (0.48, 7.14)	7	
	5−25 ppm-yr	1.33 (0.27, 6.59)	3	
	>25 ppm-yr	1.67 (0.40, 7.00)	5	
	Females, cumulative exposure	2.81 (0.25, 31.10)	2	
	0	1.0[a]		
	<5 ppm-yr	3.99 (0.25, 63.94)	1	
	5−25 ppm-yr	9,.59 (0.60, 154.14)	1	
	>25 ppm-yr		0	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	TCE exposed workers	Not reported		
	Unexposed workers	Not reported		
Deaths reported to among GE pension fund (Pittsfield, MA)		0.95 (0.1, 3.17)[i]	13	Greenland et al., 1994
Cardboard manufacturing workers, Atlanta area, GA		Not reported		Sinks et al., 1992
U. S. Coast Guard employees				Blair et al., 1989
	Marine inspectors	0.72 (0.09, 2.62)	2	
	Noninspectors	0.74 (0.09, 2.68)	2	
Aircraft manufacturing plant employees (Italy)				Costa et al., 1989
	All subjects	0.21 (0.01, 1.17)	1	

Table 4-99. Summary of human studies on TCE exposure and esophageal cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Rubber Workers		Not reported[i]		Wilcosky et al., 1984
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects	1.14 (0.62, 1.92)	14	
Case-control studies				
Population of Montreal, Canada				Siemiatycki et al., 1991; Parent et al., 2000
	Any TCE exposure	0.5 (0.1, 2.5)[j]	1	
	Substantial TCE exposure	0.8 (0.1, 4.6)[j]	1	
Geographic based studies				
Residents in two study areas in Endicott, NY		0.78 (0.29, 1.70)	6	ATSDR, 2006
Residents of 13 census tracts in Redlands, CA		Not reported		Morgan and Cassidy, 2002
Finnish residents				
	Residents of Hausjarvi	Not reported		Vartiainen et al., 1993
	Residents of Huttula	Not reported		

[a]Internal referents, workers not exposed to TCE.
[b]Ritz (1999) and Zhao et al. (2005) reported relative risks for the combined site of esophagus and stomach.
[c]Sung et al. (2007) and Chang et al. (2005)—SIR for females and reflects a 10-year lag period.
[d]SIR for adenocarcinoma of the esophagus.
[e]The SIR for adenocarcinoma histologic type can not be calculated because Hansen et al. (2001) do not present expected numbers for adenocarcinoma histologic type of esophageal cancer. An approximation of the SIR for adenocarcinoma histologic type is presented using the expected number of total number of expected esophageal cancers for males (n = 1.4). The expected numbers of esophageal adenocarcinomas in males will be lower; Hansen et al. (2001) noted the proportion of adenocarcinomas among the comparable Danish male population during the later period of the study (1990−1996) as 38%. A rough approximation of the expected number of esophageal carcinomas would be 0.5 expected cases and an approximated SIR of 9.4 (3.1, 22).
[f]Proportional mortality ratio.
[g]Adjusted relative risks for >2 year exposure duration and 15 year lag from 1st exposure.
[h]No esophageal cancer deaths occurred in the referent population in Blair et al. (1998) and relative risk in could not be calculated for this reason.
[i]Odds ratio from nested case-control analysis.
[j]90% confidence interval.

Meta-analysis is not adopted as a tool for examining the body of epidemiologic evidence on esophageal cancer and TCE exposure given the absence of reported relative risk estimates in several of the high-quality studies (Axelson et al., 1994; Anttila et al., 1995; Morgan et al., 1998).

Overall, three high-quality cohort studies provide some evidence of association for esophageal cancer and TCE exposure. The finding in two of these studies of esophageal risk estimates among subjects with long employment duration were higher than those associated with

low employment duration provides additional evidence (Hansen et al., 2001; Raaschou-Nielsen et al., 2003). The cohort studies are unable to directly examine possible confounding due to suspected risk factors for esophageal cancer such as smoking, obesity and alcohol. The use of an internal referent group, similar in socioeconomic status as exposed subjects, is believed to minimize but may not completely control for possible confounding related to smoking and health status (Blair et al., 1998; its follow-up Radican et al., 2008; Zhao et al., 2005; Boice et al, 2006). Observation of a higher risk for adenocarcinoma histologic type than for a combined category of esophageal cancer in Raaschou-Nielsen et al. (2003) also suggests minimal confounding from smoking. Smoking is not identified as a possible risk factor for the adenocarcinoma histologic type of esophageal cancer but is believed a risk factor for squamous cell histologic type. Furthermore, the magnitude of lung cancer risk in Raaschou-Nielsen et al. (2003) suggests a high smoking rate is unlikely. The lack of association with overall TCE exposure and the absence of exposure-response patterns in the other studies of TCE exposure may reflect limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. These studies do not provide evidence against an association between TCE exposure and esophageal cancer.

4.9.2. Bladder Cancer

Twenty-five epidemiologic studies present risk estimates for bladder cancer (Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Mallin, 1990; Siemiatycki, 1991; Sinks et al., 1992; Axelson et al., 1994; Greenland et al., 1994; Anttila et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999, 2006; Pesch et al., 2000b; Hansen et al., 2001; Cassidy and Morgan, 2002; Chang et al., 2003, 2005; Raaschou-Nielsen et al., 2003; ATSDR, 2004, 2006; Zhao et al., 2005; Sung et al., 2007; Radican et al., 2008). Table 4-100 presents risk estimates for TCE exposure and bladder cancer observed in cohort, case-control, and geographic based studies. Thirteen studies, all either cohort or case-control studies, which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on job-exposure matrices or biomarker monitoring) or which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review, reported relative risk estimates for bladder or urothelial cancer between 0.6 (Siemiatycki, 1991) and 1.7 (Boice et al., 2006) and overall TCE exposure. Relative risk estimates were generally based on small numbers of cases or deaths, except for one study (Raaschou-Nielsen et al., 2004), with the result of wide confidence intervals on the estimates. Of high-quality studies, two reported statistically significant elevated bladder or urothelial cancer risks with the highest cumulative TCE exposure category (2.71, 95% CI: 1.10, 6.65 [Morgan et al., 1998]; 1.8, 95% CI: 1.2, 2.7 [Pesch et al., 2000b]) and five presented risk estimates and categories of increasing cumulative TCE exposure (Blair et al., 1998; Morgan

et al., 1998; Pesch et al., 2000b; Zhao et al., 2005; Radican et al., 2008). Risk estimates in Morgan et al. (1998), Pesch et al. (2000b), and Zhao et al. (2005) appeared to increase with increasing cumulative TCE exposure with the *p*-value for trend of 0.07 in Zhao et al. (2005), the only study to present a formal statistical test for linear trend. Risk estimates did not appear to either increase or decrease with increasing cumulative TCE exposure in Blair et al. (1998) or its update Radican et al. (2008), which added another 10 years of follow-up. Twelve additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease statistical power and study sensitivity (Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Mallin, 1990; Sinks et al., 1992; Cassidy and Morgan, 2002; Chang et al., 2003, 2005; ATSDR, 2004, 2006; Sung et al., 2007).

Meta-analysis is not adopted as a tool for examining the body of epidemiologic evidence on bladder cancer and TCE.

Overall, three high-quality cohort or case-control studies provide some evidence of association for bladder or urothelial cancer and high cumulative TCE exposure (Morgan et al., 1998; Pesch et al., 2000b; Zhao et al., 2005). The case-control study of Pesch et al. (2000b) adjusted for age, study center, and cigarette smoking, with a finding of a statistically significant risk estimate between urothelial cancer and the highest TCE exposure category. Cancer cases in this study are of several sites, bladder, ureter, and renal pelvis, and grouping different site-specific cancers with possible etiologic heterogeneity may introduce misclassification bias. The cohort studies are unable to directly examine possible confounding due to suspected risk factors for esophageal cancer such as smoking, obesity, and alcohol. The use of an internal referent group, similar in socioeconomic status as exposed subjects, by Morgan et al. (1998) and Zhao et al. (2005) is believed to minimize but may not completely control for possible confounding related to smoking and health status. The lack of association with overall TCE exposure in other studies and the absence of exposure-response patterns with TCE exposure in Blair et al. (1998) and Radican et al. (2008) may reflect limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. These studies do not provide evidence against an association between TCE exposure and bladder cancer.

Table 4-100. Summary of human studies on TCE exposure and bladder cancer

1

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al., 2005
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00[a]	20	
	Medium cumulative TCE score	1.54 (0.81, 2.92) [b]	19	
	High TCE score	1.98 (0.93, 4.22) [b]	11	
	p for trend	$p = 0.069$		
	TCE, 20 yrs exposure lag			
	Low cumulative TCE score	1.00[a]	20	
	Medium cumulative TCE score	1.76 (0.61, 5.10) [c]	20	
	High TCE score	3.68 (0.87, 15.5) [c]	10	
	p for trend	$p = 0.064$		
All employees at electronics factory (Taiwan)				
	Males	Not reported		Sung et al., 2007
	Females	0.34 (0.07, 1.00)	10	
	Males	1.06 (0.45, 2.08) [d]	8	Chang et al., 2005
	Females	1.09 (0.56, 1.91) [d]	12	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure, all subjects	1.1 (0.92, 1.21)	220	
	Any exposure, males	1.0 (0.89, 1.18)	203	
	Any exposure, females	1.6 (0.93, 2.57)	17	
Biologically-monitored Danish workers		1.0 (0.48, 1.86)	10	Hansen et al., 2001
	Any TCE exposure, males	1.1 (0.50, 2.0)	10	
	Any TCE exposure, females	0.5 expected	0	
Aircraft maintenance workers from Hill Air Force Base				Blair et al., 1998
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.7 (0.6, 4.4)	13	
	5−25 ppm-yr	1.7 (0.6, 4.9)	9	
	>25 ppm-yr	1.4 (0.5, 4.1)	9	

1

Table 4-100. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.1 (0.1, 10.8)	1	
	5−25 ppm-yr		0	
	>25 ppm-yr	1.0 (0.1, 9.1)	1	
Biologically-monitored Finnish workers				Anttila et al., 1995
	All subjects	0.82 (0.27, 1.90)	5	
Biologically-monitored Swedish workers				Axelson et al., 1994
	Any TCE exposure, males	1.02 (0.44, 2.00)	8	
	Any TCE exposure, females	Not reported		
Cohort and PMR studies-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	1.66 (0.54, 3.87)	5	Boice et al., 2006
	Any exposure to TCE	Not reported		Zhao et al., 2005
	Low cumulative TCE score	1.00[a]	8	
	Med cumulative TCE score	1.27 (0.43, 3.73) [b]	6	
	High TCE score	1.15 (0.29, 4.51) [b]	3	
	p for trend	$p = 0.809$		
	TCE, 20 yrs exposure lag			
	Low cumulative TCE score	1.00[a]	8	
	Medium cumulative TCE score	0.95 (0.15, 6.02) [c]	7	
	High TCE score	1.85 (0.12, 27.7) [c]	2	
	p for trend	$p = 0.533$		
View-Master employees				ATSDR, 2004
	Males	1.22 (0.15, 4.40)		
	Females	0.78 (0.09, 2.82)		
United States uranium-processing workers (Fernald)				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration	Not reported		
	Moderate TCE exposure, >2 yrs duration	Not reported		
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure	0.55 (0.18, 1.28)	5	
	Routine-intermittent[a]	Not reported		

Table 4-100. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	1.36 (0.59, 2.68)	8	
	Low intensity (<50 ppm)	0.51 (0.01, 2.83)	1	
	High intensity (>50 ppm)	1.79 (0.72, 3.69)	7	
	TCE subcohort (Cox Analysis)			
	Never exposed	1.0[a]		
	Ever exposed	2.05 (0.86, 4.85)[e]	8	
	Peak			
	No/low	1.0[a]		
	Medium/high	1.41 (0.52, 3.81)	5	
	Cumulative			
	Referent	1.0[a]		
	Low	0.69 (0.09, 5.36)	1	
	High	2.71 (1.10, 6.65)	7	
Aircraft maintenance workers (Hill AFB, UT)				Blair et al., 1998
	TCE subcohort	1.2 (0.5, 2.9)[a]	17	
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	1.8 (0.5, 6.2)	7	
	5−25 ppm-yr	2.1 (0.6, 8.0)	5	
	>25 ppm-yr	1.0 (0.2, 5.1)	3	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr		0	
	5−25 ppm-yr		0	
	>25 ppm-yr	0.8 (0.1, 7.5)	1	
	TCE subcohort	0.80 (0.41, 1.58)	25	Radican et al., 2008
	Males, cumulative exposure	1.05 (0.47, 2.35)	24	
	0	1.0[a]		
	<5 ppm-yr	0.96 (0.37, 2.51)	9	
	5−25 ppm-yr	1.77 (0.70, 4.52)	10	
	>25 ppm-yr	0.67 (0.15, 2.95)	5	

Table 4-100. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Females, cumulative exposure	0.22 (0.03, 1.83)	1	
	0	1.0[a]		
	<5 ppm-yr		0	
	5−25 ppm-yr	2.86 (0.27, 29.85)	1	
	>25 ppm-yr		0	
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	TCE exposed workers	Not reported		
	Unexposed workers	Not reported		
Deaths reported to GE pension fund (Pittsfield, MA)		0.85 (0.32, 2.23)[f]	20	Greenland et al., 1994
Cardboard manufacturing workers, Atlanta area, GA				Sinks et al., 1992
		0.3 (0.0, 1.6)	1	
U. S. Coast Guard employees				Blair et al., 1989
	Marine inspectors	0.50 (0.06, 1.79)	2	
	Noninspectors	0.90 (0.18, 2.62)	3	
Aircraft manufacturing plant employees (Italy)				Costa et al., 1989
	All subjects	0.74 (0.30, 1.53)	7	
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects	1.26 (0.74, 2.03)	17	
Lamp manufacturing workers (GE)		0.93 (0.19, 2.72)	3	Shannon et al., 1988
Case-control studies				
Population of 5 regions in Germany				Pesch et al., 2000b
	Any TCE exposure	Not reported		
	Males	Not reported		
	Females	Not reported		
	Males			
	Medium	0.8 (0.6, 1.2)[g]	47	
	High	1.3 (0.8, 1.7)[g]	74	
	Substantial	1.8 (1.2, 2.7)[g]	36	
Population of Montreal, Canada				Siemiatycki, 1991; Siemiatycki et al., 1994
	Any TCE exposure	0.6 (0.3, 1.2)	8	
	Substantial TCE exposure	0.7 (0.3, 1.6)	5	
Geographic based studies				
Residents in two study areas in Endicott, NY				ATSDR, 2006
		0.71 (0.38, 1.21)	13	

Table 4-100. Summary of human studies on TCE exposure and bladder cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Residents of 13 census tracts in Redlands, CA				Morgan and Cassidy, 2002
		0.98 (0.71, 1.29) [h]	82	
Finnish residents				Vartiainen et al., 1993
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		
Residents of 9 county area in Northwestern Illinois				Mallin, 1990
	All zip codes in study area			
	Males	1.4 (1.1, 1.9)	47	
	Females	1.8 (1.2, 2.7)	21	
	Cluster community			
	Males	1.7 (1.1, 2.6)	21	
	Females	2.6 (1.2, 4.7)	10	
	Adjacent community			
	Males	1.2 (0.6, 2.0)	12	
	Females	1.6 (0.5, 3.8)	5	
	Remainder of zip code areas			
	Males	1.4 (0.8, 2.2)	14	
	Females	1.4 (0.5, 3.0)	6	

[a]Internal referents, workers not exposed to TCE.
[b]Relative risk estimates for TCE exposure after adjustment for 1st employment, socioeconomic status, and age at event.
[c]Relative risk estimates for TCE exposure after adjustment for 1st employment, socioeconomic status, age at event, and all other carcinogen exposures, including hydrazine.
[d]Chang et al. (2005) and Costa et al. (1989) report estimated risks for a combined site of all urinary organ cancers.
[e]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex and decade (Environmental Health Strategies, 1997).
[f]Odds ratio from nested case-control analysis.
[g]Odds ratio for urothelial cancer, a category of bladder, ureter, and renal pelvis cancers) and cumulative TCE exposure, as assigned using a job-task-exposure matrix (JTEM) approach (Pesch et al., 2000b).
[h]99% confidence interval.

4.9.3. Central Nervous System and Brain Cancers

Brain cancer is examined in most cohort studies and in one case-control study (Garabrant et al., 1988; Blair et al., 1989; Costa et al., 1989; Greenland et al., 1994; Heineman et al., 1994; Anttila et al., 1995; Henschler et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999, 2006; Ritz, 1999; Hansen et al., 2001; Chang et al., 2003, 2005; Raaschou-Nielsen et al.,

2003; Zhao et al., 2005; Sung et al., 2007; Clapp and Hoffman, 2008; Radican et al., 2008). Overall, these epidemiologic studies do not provide strong evidence for or against association between TCE and brain cancer in adults (see Table 4-101). Relative risk estimates in well designed and conducted cohort studies, Axelson et al. (1994), Anttila et al. (1995), Blair et al. (1998), its follow-up reported in Radican et al. (2008), Morgan et al. (1998), Boice et al. (1999), Zhao et al. (2005), and Boice et al. (2006), are near a risk of 1.0 and imprecise, confidence intervals all include a risk estimate of 1.0. All studies except Raaschou-Nielsen et al. (2003), observations are based on few events and lowered statistical power. Bias resulting from exposure misclassification is likely in these studies, although of a lower magnitude compared to other cohort studies identified in Table 4-101, and may partly explain observations. Exposure misclassification is also likely in the case-control study of occupational exposure of Heineman et al. (1994) who do not report association with TCE exposure.

Three geographic-based studies and one case-control study examined childhood brain cancer (AZ DHS, 1990, 1995; De Roos et al., 2001; Morgan and Cassidy, 2002; ATSDR, 2006). The strongest study, De Roos et al. (2001), a population case-control study which examined paternal exposure, used expert judgment to evaluate the probably of TCE exposure from self-reported information in an attempt to reduce exposure misclassification bias. The odds ratio estimate in this study was 0.9 (95% CI: 0.3, 2.5). Like many population case-control studies, a low prevalence of TCE exposure was found, only 9 fathers were identified with probable TCE exposure by the industrial hygiene review, and greatly impacted statistical power. There is some concern for childhood brain cancer and organic solvent exposure based on Peters et al. (1981) whose case-control study of childhood brain cancer reported to the Los Angeles County Cancer Surveillance Program observed a high odds ratio estimate for paternal employment in the aircraft industry (OR: ∞, $p < 0.001$). This study does not present an odds ratio for TCE exposure only although it did identify two of the 14 case and control fathers with previous employment in the aircraft industry reported exposure to TCE.

4.10. SUSCEPTIBLE LIFESTAGES AND POPULATIONS

Variation in response among segments of the population may be due to age, genetics, and ethnicity, as well as to differences in lifestyle, nutrition, and disease status. These could be potential risk factors that play an important role in determining an individual's susceptibility and sensitivity to chemical exposures. Studies on TCE toxicity in relation to some of these risk factors including lifestage, gender, genetics, race/ethnicity, pre-existing health status, and lifestyle are discussed below. Others have also reviewed factors related to human variability and their potential for susceptibility to TCE (Barton et al., 1996; Clewell et al., 2000; Davidson and Beliles, 1991; NRC, 2006; Pastino et al., 2000).

Table 4-101. Summary of human studies on TCE exposure and brain cancer

1

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al., 2005
	Any exposure to TCE	Not reported		
	Low cumulative TCE score	1.00[a]	7	
	Medium cumulative TCE score	0.46 (0.09, 2.25) [b]	2	
	High TCE score	0.47 (0.06, 3.95) [b]	1	
	p for trend	*p* = 0.382		
All employees at electronics factory (Taiwan)				
	Males	Not reported		Sung et al., 2007
	Females	1.07 (0.59, 1.80) [c]		
	Males	0.40 (0.05, 1.46)	2	Chang et al., 2005
	Females	0.97 (0.54, 1.61)	15	
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure, all subjects	1.0 (0.84, 1.24)	104	
	Any exposure, males	1.0 (0.76, 1.18)	85	
	Any exposure, females	1.1 (0.67, 1.74)	19	
Biologically-monitored Danish workers		0.3 (0.01, 1.86)	1	Hansen et al., 2001
	Any TCE exposure, males	0.4 (0.01, 2.1)	1	
	Any TCE exposure, females	0.5 expected	0	
Aircraft maintenance workers from Hill Air Force Base				Blair et al., 1998
	TCE subcohort	Not reported		
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	2.0 (0.2, 19.7)	3	
	5−25 ppm-yr	3.9 (0.4, 34.9)	4	
	>25 ppm-yr	0.8 (0.1, 13.2)	1	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr		0	
	5−25 ppm-yr		0	
	>25 ppm-yr		0	
Biologically-monitored Finnish workers				Anttila et al., 1995
	All subjects	1.09 (0.50, 2.07)	9	
	Mean air-TCE (Ikeda extrapolation)			
	<6 ppm	1.52 (0.61, 3.13)	7	
	6+ ppm	0.76 (0.01, 2.74)	2	
Biologically-monitored Swedish workers				Axelson et al., 1994
	Any TCE exposure, males	Not reported		
	Any TCE exposure, females	Not reported		

1

Table 4-101. Summary of human studies on TCE exposure and brain cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort and PMR studies-mortality				
Computer manufacturing workers (IBM), NY				Clapp and Hoffman, 2008
	Males	1.90 (0.52, 4.85)	4	
	Females		0	
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	0.81 (0.17, 2.36)	3	Boice et al., 2006
	Any exposure to TCE	Not reported		Zhao et al., 2005
	Low cumulative TCE score	1.00[a]	12	
	Medium cumulative TCE score	0.42 (0.12, 1.50)	3	
	High TCE score	0.83 (0.23, 3.08)	3	
	p for trend	$p = 0.613$		
View-Master employees				ATSDR, 2004
	Males	Not reported		
	Females	Not reported		
All employees at electronics factory (Taiwan)				Chang et al., 2003
	Males	0.96 (0.01, 5.36)	1	
	Females	0.96 (0.01, 5.33)	1	
United States uranium-processing workers (Fernald)				Ritz, 1999
	Any TCE exposure	Not reported		
	Light TCE exposure, >2 yrs duration, 0 lag	1.81 (0.49, 6.71) [d]	6	
	Moderate TCE exposure, >2 yrs duration, 0 lag	3.26 (0.37, 28.9) [d]	1	
	Light TCE exposure, >5 yrs duration, 15 yr lag	5.41 (0.87, 33.9) [d]	3	
	Moderate TCE exposure, >5 yrs duration, 15 yr lag	14.4 (1.24, 167) [d]	1	
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine exposure	0.54 (0.15, 1.37)	4	
	Routine-intermittent[a]	Not presented		
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	0.99 (0.64, 1.47)	4	
	Low intensity (<50 ppm)[d]	0.73 (0.09, 2.64)	2	
	High intensity (>50 ppm) [d]	0.44 (0.05, 1.58)	2	
Aircraft maintenance workers (Hill AFB, Utah)				Blair et al., 1998
	TCE subcohort	0.8 (0.2, 2.2) [a]	11	

Table 4-101. Summary of human studies on TCE exposure and brain cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Males, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr	0.7 (0.7, 3,3)	3	
	5−25 ppm-yr	2.0 (0.5, 8.4)	5	
	>25 ppm-yr	0.9 (0.2, 4.4)	2	
	Females, cumulative exposure			
	0	1.0[a]		
	<5 ppm-yr		0	
	5−25 ppm-yr		0	
	>25 ppm-yr		0	
	TCE subcohort	1.02 (0.39, 2.67)	17	Radican et al., 2008
	Males, cumulative exposure	1.26 (0.43, 3.75)	17	
	0	1.0[a]		
	<5 ppm-yr	1.46 (0.44, 4.86)	8	
	5−25 ppm-yr	1.74 (0.49, 6.16)	6	
	>25 ppm-yr	0.66 (0.15, 2.95)	3	
	Females, cumulative exposure		0	
	0			
	<5 ppm-yr			
	5−25 ppm-yr			
	>25 ppm-yr			
Cardboard manufacturing workers in Arnsburg, Germany				Henschler et al., 1995
	TCE exposed workers	3.70 (0.09, 20.64)	1	
	Unexposed workers	9.38 (1.93, 27.27)	3	
Deaths reported to GE pension fund (Pittsfield, MA)		0.93 (0.32, 2.69)[e]	16	Greenland et al., 1994
Cardboard manufacturing workers, Atlanta area, GA				Sinks et al., 1992
		Not reported		
U. S. Coast Guard employees				Blair et al., 1989
	Marine inspectors	1.70 (0.55, 3.95)	5	
	Noninspectors	1.36 (0.44, 3.17)	5	
Aircraft manufacturing plant employees (Italy)				Costa et al., 1989
	All subjects	0.79 (0.16, 2.31)	3	
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects	0.78 (0.42, 1.34)	16	
Case-control studies				
Children's Cancer Group/Pediatric Oncology Group				De Roos et al., 2001
	Any TCE exposure	1.64 (0.95, 2.84)	37	
	Neuroblastoma, ≤15 yrs age			

Table 4-101. Summary of human studies on TCE exposure and brain cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Paternal TCE exposure			
	Self-reported exposure	1.4 (0.7, 2.9)	22	
	IH assignment of probable exposure	0.9 (0.3, 2.5)	9	
Population of So. LA, NJ, Philadelphia PA				Heineman et al., 1994
	Any TCE exposure	1.1 (0.8, 1.6)	128	
	Low exposure	1.1 (0.7, 1.7)	27	
	Medium exposure	1.1 (0.6, 1.8)	42	
	High exposure	1.1 (0.5, 2.8)	12	
	p for trend	0.45		
Geographic based studies				
Residents in two study areas in Endicott, NY				ATSDR, 2006
	Brain/CNS, ≤19 yrs of age	Not reported	<6	
Residents of 13 census tracts in Redlands, CA				Morgan and Cassidy, 2002
	Brain/CNS, <15 yrs of age	1.05 (0.24, 2.70) [f]	6	
Resident of Tucson Airport Area, AZ				AZ DHS, 1990, 1995
	Brain/CNS, ≤19 yrs of age			
	1970−1986	0.84 (0.23, 2.16)	3	
	1987−1991	0.78 (0.26, 2.39)	2	

[a]Internal referents, workers not exposed to TCE.
[b]Relative risks for TCE exposure after adjustment for 1st employment, socioeconomic status, and age at event.
[c]Standardized incidence ratio from analyses lagging exposure 10 years prior to end of follow-up or date of incident cancer.
[d]Relative risks for TCE exposure after adjustment for time since 1st hired, external and internal radiation dose, and same chemical at a different level.
[e]Odds ratio from nested case-control analysis.
[f]99% confidence interval.

4.10.1. Lifestages

Individuals of different lifestages are physiologically, anatomically, and biochemically different. Early (infants and children) and later (the elderly) lifestages differ greatly from adulthood in body composition, organ function, and many other physiological parameters that can influence the toxicokinetics of chemicals and their metabolites in the body (ILSI, 1992). The limited data on TCE exposure suggest that these segments of the population—particularly individuals in early lifestages—may have greater susceptibility than does the general population. This section presents and evaluates the pertinent published literature available to assess how individuals of differing lifestages may respond differently to TCE.

4.10.1.1. *Early Lifestages*

4.10.1.1.1. *Early lifestage-specific exposures.* Section 2.4 describes the various exposure pathways of concern for TCE. For all postnatal lifestages, the primary exposure routes of concern include inhalation and contaminated drinking water. In addition, there are exposure pathways to TCE are unique to early lifestages. Fetal and infant exposure to TCE can occur through placental transfer and breast milk consumption if the mother has been exposed, and could potentially increase overall TCE exposure. Placental transfer of TCE has been demonstrated in humans (Beppu, 1968; Laham, 1970), rats (Withey and Karpinski, 1985), mice (Ghantous et al., 1986), rabbits (Beppu, 1968), and sheep and goats (Helliwell and Hutton, 1950). Similarly, TCE has been found in breast milk in humans (Fisher et al., 1997; Pellizzari et al., 1982), goats (Hamada and Tanaka, 1995), and rats (Fisher et al., 1990). Pellizzari et al. (1982) conducted a survey of environmental contaminants in human milk, using samples from cities in the northeastern region of the United States and one in the southern region and detected TCE in 8 milk samples taken from 42 lactating women. No details of times postpartum, milk lipid content or TCE concentration in milk or blood were reported. Fisher et al. (1997) predicted that a nursing infant would consume 0.496 mg TCE during a 24-hour period. In lactating rats exposed to 600 ppm (3,225 mg/m^3) TCE for 4 hours resulted in concentrations of TCE in milk of 110 µg/mL immediately following the cessation of exposure (Fisher et al., 1990).

Direct childhood exposures to TCE from oral exposures may also occur. A contamination of infant formula resulted in levels of 13 ppb (Fan, 1988). Children consume high levels of dairy products, and TCE has been found in butter and cheese (Wu and Schaum, 2000). In addition, TCE has been found in food and beverages containing fats such as margarine (Wallace et al., 1984), grains and peanut butter (Wu and Schaum, 2000), all of which children consume in high amounts. A number of studies have examined the potential adverse effects of childhood exposure to drinking water contaminated with TCE (ATSDR, 1998, 2001; Bernad et al., 1987; Bove, 1996; Bove et al., 1995; Burg and Gist, 1999; Goldberg et al., 1990; Lagakos et al., 1986; Rodenbeck et al., 2000; Sonnenfeld et al., 2001; White et al., 1997; see Section 4.10.2.1). TCE in residential water may also be a source of dermal or inhalation exposure during bathing and showering (Fan, 1988; Franco et al., 2007; Giardino and Andelman, 1996; Lee et al., 2002; Weisel and Jo, 1996; Wu and Schaum, 2000); it has been estimated that showering and bathing scenarios in water containing 3-ppm TCE, a child of 22 kg receives a higher dose (about 1.5 times) on a mg/kg basis than a 70 kg adult (Fan, 1988).

Direct childhood inhalation exposure to TCE have been documented in both urban and rural settings. A study of VOCs measured personal, indoor and outdoor TCE in 284 homes, with 72 children providing personal measures and time-activity diaries (Adgate et al., 2004a). The intensive-phase of the study found a mean personal level of 0.8 µg/m^3 and mean indoor and

outdoor levels of 0.6 μg/m^3, with urban homes have significantly higher indoor levels of TCE than nonurban homes (t = 2.3, p = 0.024) (Adgate et al., 2004a). A similar study of personal, indoor and outdoor TCE was conducted in two inner-city elementary schools as well as in the homes of 113 children along with time-activity diaries, and found a median a median personal level of 0.3 μg/m^3, a median school indoor level of 0.2 μg/m^3, a median home indoor level of 0.3 μg/m^3, a median outdoor level of 0.3 μg/m^3 in the winter, with slightly lower levels in the spring (Adgate et al., 2004b). Studies from Leipzig, Germany measured the median air level of TCE in children's bedrooms to be 0.42 μg/m^3 (Lehmann et al., 2001) and 0.6 μg/m^3 (Lehmann et al., 2002). A study of VOCs in Hong Kong measured air levels in schools, including an 8-hour average of 1.28 μg/m^3, which was associated with the lowest risk of cancer in the study (Guo et al., 2004). Another found air TCE levels to be highest in school/work settings, followed by outside, in home, in other, and in transit settings (Sexton et al., 2007). Measured indoor air levels ranged from 0.18−140 μg/m^3 for children exposed through vapor intrusion from soil vapor (ATSDR, 2006). Contaminated soil may be a source of either dermal or ingestion exposure of TCE for children (Wu and Schaum, 2000).

Additional TCE exposure has also been documented to have occurred during medical procedures. TCE was used in the past as an anesthetic during childbirth (Beppu, 1968; Phillips and Macdonald, 1971) and surgery during childhood (Jasinka, 1965). These studies are discussed in more detail in Section 4.8.3.1.1. In addition, the TCE metabolite chloral hydrate has been used as an anesthetic for children for CAT scans (Steinberg, 1993).

Dose received per body weight for 3-ppm TCE via oral, dermal, dermal plus inhalation, and bathing scenarios was estimated for a 10-kg infant, a 22-kg child, and a 70-kg adult (Fan, 1988; see Table 4-102). For the oral route (drinking water), an infant would receive a higher daily dose than a child, and the child more than the adult. For the dermal and dermal plus inhalation route, the child would receive more than the adult. For the bathing scenario, the infant and child would receive comparable amounts, more than the adult.

4.10.1.1.2. ***Early lifestage-specific toxicokinetics.*** Chapter 3 describes the toxicokinetics of TCE. However, toxicokinetics in developmental lifestages are distinct from toxicokinetics in adults (Benedetti et al., 2007; Ginsberg et al., 2002, 2004a, 2004b; Hattis et al., 2003) due to, for example, altered ventilation rates, percent adipose tissue, and metabolic enzyme expression. Early lifestage-specific information is described below for absorption, distribution, metabolism, and excretion, followed by available early lifestage-specific PBPK models.

Table 4-102. Estimated lifestage-specific daily doses for TCE in water*

	Body weight		
	Infant (10 kg)	**Child (22 kg)**	**Adult (70 kg)**
Drinking water	0.3 mg/kg	0.204 mg/kg	0.086 mg/kg
Showering—dermal	-	0.1 mg/kg	0.064 mg/kg
Showering—dermal and inhalation	-	0.129 mg/kg	0.083 mg/kg
Bathing—15 min	-	0.24 mg/kg	0.154 mg/kg
Bathing—5 min	0.08 mg/kg	0.08 mg/kg	0.051 mg/kg

*Adapted from Fan (1988).

4.10.1.1.2.1. *Absorption*. As discussed in Section 3.1, exposure to TCE may occur via inhalation, ingestion, and dermal absorption. In addition, prenatal exposure may result in absorption via the transplacental route. Exposure via inhalation is proportional to the ventilation rate, duration of exposure, and concentration of expired air, and children have increased ventilation rates per kg body weight compared to adults, with an increased alveolar surface area per kg body weight for the first two years (U.S. EPA, 2008). It is not clear to what extent dermal absorption may be different for children compared to adults; however, infants have a 2-fold increase in surface area compared to adults, although similar permeability (except for premature babies) compared to adults (U.S. EPA, 2008).

4.10.1.1.2.2. *Distribution*. Both human and animal studies provide clear evidence that TCE distributes widely to all tissues of the body (see Section 3.2). For lipophilic compounds such as TCE, percentage adipose tissue, which varies with age, will affect absorption and retention of the absorbed dose. Infants have a lower percentage of adipose tissue per body weight than adults, resulting in a higher concentration of the lipophilic compound in the fat of the child (NRC, 1993).

During pregnancy of humans and experimental animals, TCE is distributed to the placenta (Beppu, 1968; Ghantous et al., 1986; Helliwell and Hutton, 1950; Laham, 1970; Withey and Karpinski, 1985). In humans, TCE has been found in newborn blood after exposure to TCE during childbirth with ratios of concentrations in fetal:maternal blood ranging from approximately 0.5 to approximately 2 (Laham, 1970). In childhood, blood levels concentrations of TCE were found to range from 0.01−0.02 ng/mL (Sexton et al., 2005). Pregnant rats exposed to TCE vapors on GD 17 resulted in concentrations of TCE in fetal blood approximately one-

third the concentration in corresponding maternal blood, and was altered based upon the position along the uterine horn (Withey and Karpinski, 1985). TCE has also been found in the organs of prenatal rabbits including the brain, liver, kidneys and heart (Beppu, 1968). Rats prenatally exposed to TCE had increased levels measured in the brain at PND10, compared to rats exposed as adults (Rodriguez et al., 2007). TCE can cross the blood-brain barrier during both prenatal and postnatal development, and may occur to a greater extent in younger children. It is also important to note that it has been observed in mice that TCE can cycle from the fetus into the amniotic fluid and back to the fetus (Ghantous et al., 1986).

Studies have examined the differential distribution by age to a mixture of six VOCs including TCE to children aged 3−10 years and adults aged 20−82 years old (Mahle et al., 2007) and in rats at PND10, 2 months (adult), and 2 years (aged) (Mahle et al., 2007; Rodriguez et al., 2007). In humans, the blood:air partition coefficient for male or female children was significantly lower compared to adult males (Mahle et al., 2007). In rats, the difference in tissue:air partition coefficients increased with age (Mahle et al., 2007). Higher peak concentrations of TCE in the blood were observed in the PND10 rat compared to the adult rat after inhalation exposure, likely due to the lower metabolic capacity of the young rats (Rodriguez et al., 2007).

4.10.1.1.2.3. *Metabolism*. Section 3.3 describes the enzymes involved in the metabolism of TCE, including CYP and GST. Expression of these enzymes changes during various stages of fetal development (Dorne et al., 2005; Hakkola et al., 1996a, b, 1998a, b; Hines and McCarver, 2002; Shao et al., 2007; van Lieshout et al., 1998) and during postnatal development (Blake et al., 2005; Dorne et al., 2005; Tateishi et al., 1997), and may result in altered susceptibility.

Expression of CYP enzymes have been shown to play a role in decreasing the metabolism of TCE during pregnancy in rats, although metabolism increased in young rats (3-week-old) compared to adult rats (18-week-old) (Nakajima et al., 1992a). For TCE, CYP2E1 is the main metabolic CYP enzyme, and expression of this enzyme has been observed in humans in prenatal brain tissue at low levels beginning at 8-weeks gestation and increasing throughout gestation (Brzezinski et al., 1999). Very low levels of CYP2E1 have been detected in some samples fetal liver during the second trimester (37% of samples) and third trimester (80% of samples) (Carpenter et al., 1996; Johnsrud et al., 2003), although hepatic expression surges immediately after birth in most cases (Johnsrud et al., 2003; Vieira et al., 1996) and in most infants reaches adult values by 3 months of age (Johnsrud et al., 2003; Vieira et al., 1996).

Although there is some uncertainty as to which GST isoforms mediate TCE conjugation, it should be noted that their expression changes with fetal development (McCarver and Hines, 2002; Raijmakers et al., 2001; van Lieshout et al., 1998).

4.10.1.1.2.4. *Excretion*. The major processes of excretion of TCE and its metabolites are discussed in Section 3.4, yet little is know about whether there are age-related differences in excretion of TCE. The major pathway for elimination of TCE is via exhalation, and its metabolites via urine and feces, and it is known that renal processes are not mature until about 6 months of age (NRC, 1993). Only one study was identified that measured TCE or its metabolites in exhaled breath and urine in a 17-year old who ingested a large quantity of TCE (Brüning et al., 1998). TCE has also been measured in the breast milk in lactating women (Fisher et al., 1997; Pellizzari et al., 1982), goats (Hamada and Tanaka, 1995), and rats (Fisher et al., 1990).

4.10.1.1.2.5. *Physiologically-based pharmacokinetic (PBPK) models*. Early lifestage-specific information regarding absorption, distribution, metabolism, and excretion needs to be considered for a child-specific and chemical-specific PBPK model. To adequately address the risk to infants and children, age-specific parameters for these values should be used in PBPK models that can approximate the internal dose an infant or child receives based on a specific exposure level (see Section 3.5).

Fisher et al. developed PBPK models to describe the toxicokinetics of TCE in the pregnant rat (Fisher et al., 1989), lactating rat and nursing pup (Fisher et al., 1990). The prenatal study demonstrates that approximately two-thirds of maternal exposure to both TCE and TCA reached the fetus after maternal inhalation, gavage, or drinking water exposure (Fisher et al., 1989). After birth, only 2% of maternal exposure to TCE reaches the pup; however, 15% and 30% of maternal TCA reaches the pup after maternal inhalation and drinking water exposure, respectively (Fisher et al., 1990). One analysis of PBPK models examined the variability in response to VOCs including TCE between adults and children, and concluded that the intraspecies uncertainty factor for pharmacokinetics is sufficient to capture variability between adults and children (Pelekis et al., 2001).

4.10.1.1.3. *Early lifestage-specific effects.* Although limited data exist on TCE toxicity as it relates to early lifestages, there is enough information to discuss the qualitative differences. In addition to the evidence described below, Section 4.8 contains information reproductive and developmental toxicity. In addition, Sections 4.3 on neurotoxicity and Section 4.6 on immunotoxicity characterize a wide array of postnatal developmental effects.

4.10.1.1.3.1. *Differential effects in early lifestages*. There are a few adverse health outcomes, in particular birth defects, which are observed only after early lifestage exposure to TCE.

Birth Defects. A summary of structural developmental outcomes that have been associated with TCE exposures is presented in Sections 4.8.2.3. In particular, cardiac birth defects have been observed after exposure to TCE in humans (ATSDR, 2006; Goldberg et al., 1990; Lagakos et al., 1986; Yauck et al., 2004), rodents (Dawson et al., 1990, 1993; Johnson et al., 1998a, b, 2003, 2005; Smith et al., 1989, 1992), and chicks (Bross et al., 1983; Loeber et al., 1988; Boyer et al., 2000; Drake et al., 2006a, b; Mishima et al., 2006; Rufer et al., 2008). However, it is notable that cardiac malformations were not observed in a number of other studies in humans (Lagakos et al., 1986; Taskinen et al., 1989; Tola et al., 1980), rodents (Carney et al., 2006; Coberly et al., 1992; Cosby and Dukelow, 1992; Dorfmueller et al., 1979; Fisher et al., 2001; Hardin et al., 1981; Healy et al., 1982; Narotsky and Kavlock, 1995; Narotsky et al., 1995; Schwetz et al., 1975), and rabbits (Hardin et al., 1981). See Section 4.8.2.3.2 for further discussion on cardiac malformations.

Structural CNS birth defects were observed in humans (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). In addition, a number of postnatal nonstructural adverse effects have been observed in humans and experimental animals following prenatal exposure to TCE. See Sections 4.3.10 and 4.8.2.3.3 for further discussion on developmental neurotoxicity.

A variety of other birth defects have been observed—including eye/ear birth anomalies in humans and rats (Lagakos et al., 1986; Narotsky et al., 1995; Narotsky and Kavlock, 1995); lung/respiratory tract disorders in humans and mice (Das and Scott, 1994; Lagakos et al., 1986); and oral cleft defects (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), kidney/urinary tract disorders, musculoskeletal birth anomalies (Lagakos et al., 1986), and anemia/blood disorders (Burg and Gist, 1999) in humans. See Section 4.8.2.3.5 for further discussion on other structural developmental outcomes. A current follow-up study of the Camp Lejeune cohort will examine birth defects and may provide additional insight (ATSDR, 2003b; GAO, 2007a, b; ATSDR, 2009).

4.10.1.1.3.2. *Susceptibility to noncancer outcomes in early lifestages*. There are a number of adverse health outcomes observed after exposure to TCE that are observed in both children and adults. Below is a discussion of differential exposure, incidence and/or severity in early lifestages compared to adulthood.

Occupational TCE poisonings via inhalation exposure resulted in an elevated percent of cases in the adolescents aged 15−19 years old (McCarthy and Jones, 1983). In addition, there is concern for intentional exposure to TCE during adolescence, including a series of deaths

involving inhaling typewriter correction fluid (King et al., 1985), a case of glue sniffing likely associated with cerebral infarction in a 12-year-old boy with a 2-year history of exposure (Parker et al., 1984), and a case of attempted suicide by ingestion of 70 mg TCE in a 17-year-old boy (Brüning et al., 1998).

4.10.1.1.3.2.1. *Neurotoxicity*. Adverse CNS effects observed after early lifestage exposure to TCE in humans include delayed newborn reflexes (Beppu, 1968), impaired learning or memory (Bernad et al., 1987, abstract; White et al., 1997); aggressive behavior (Bernad et al., 1987; Blossom et al., 2008); hearing impairment (Burg and Gist, 1999); speech impairment (Burg and Gist, 1995; White et al., 1997); encephalopathy (White et al., 1997); impaired executive and motor function (White et al., 1997); attention deficit (Bernad et al., 1987; White et al., 1997), and autism spectrum disorder (Windham et al., 2006). One analysis observed a trend for increased adversity during development, with those exposed during childhood demonstrating more deficits than those exposed during adulthood (White et al., 1997). In experimental animals, observations include decreased specific gravity of newborn brains until weaning (Westergren et al., 1984), reductions in myelination in the brains at weaning, significantly decreased uptake of 2-deoxyglucose in the neonatal rat brain, significant increase in exploratory behavior (Isaacson and Taylor, 1989; Noland-Gerbec et al., 1986; Taylor et al., 1985), decreased rearing activity (Fredriksson et al., 1993), and increased time to cross the first grid in open field testing (George et al., 1986).

Two studies addressed whether or not children are more susceptible to CNS effects (Burg et al., 1995; White et al., 1997). An analysis of three residential exposures of TCE observed speech impairments in younger children and not at any other lifestage (White et al., 1997). A national exposure registry also observed statistically significant speech impairment and hearing impairment in 0−9 year olds and no other age group (Burg et al., 1995). However, a follow-up study did not find a continued association with speech and hearing impairment in these children, although the absence of acoustic reflexes remained significant (ATSDR, 2003a). See Section 4.3 for further information on central nervous system toxicity, and Section 4.8.3.3.3 for further information on developmental neurotoxicity.

4.10.1.1.3.2.2. *Liver toxicity*. No early lifestage-specific effects were observed after TCE exposure. See Section 4.4 for further information on liver toxicity.

4.10.1.1.3.2.3. *Kidney toxicity*. Residents of Woburn, Massachusetts including 4,978 children were surveyed on residential and medical history to examine an association with contaminated wells; an association was observed for higher cumulative exposure measure and history of

kidney and urinary tract disorders (primarily kidney or urinary tract infections) and with lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia) (Lagakos et al., 1986). See Section 4.5 for further information on kidney toxicity.

4.10.1.1.3.2.4. *Immunotoxicity*. Several studies in exposure to TCE in early lifestages of humans and experimental animals were identified that assessed the potential for developmental immunotoxicity (Adams et al., 2003; Blossom and Doss, 2007; Blossom et al., 2008; Lehmann et al., 2001, 2002; Peden-Adams et al., 2006, 2008). All noted evidence of immune system perturbation except one (Lehman et al., 2001). See Section 4.6 for further information on immunotoxicity, and Section 4.8.2.3.4 for further discussion on developmental immunotoxicity.

4.10.1.1.3.2.5. *Respiratory toxicity*. Residents of Woburn, Massachusetts including 4,978 children were surveyed on residential and medical history to examine an association with contaminated wells; an association was observed for lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia) (Lagakos et al., 1986). See Section 4.7 for further information on respiratory tract toxicity.

4.10.1.1.3.3. *Susceptibility to cancer outcomes in early lifestages*. The epidemiologic and experimental animal evidence is limited regarding susceptibility to cancer from exposure to TCE during early life stages. The human epidemiological evidence is summarized above for cancer diagnosed during childhood (see Sections 4.8.2.1 and 4.8.2.3.5), including a discussion of childhood cancers of the nervous system including neuroblastoma and the immune system including leukemia (see Section 4.6.1.3). A current follow-up study of the Camp Lejeune cohort will examine childhood cancers and may provide additional insight (ATSDR, 2003b; GAO, 2007a, b; ATDSR, 2009). No studies of cancers in experimental animals in early lifestages have been observed.

4.10.1.1.3.3.1. *Total childhood cancer*. Total childhood cancers have been examined in relationship to TCE exposure (ATSDR, 2006; Morgan and Cassady, 2002). Two studies examining total childhood cancer in relation to TCE in drinking water did not observe an association. A study in Endicott, NY contaminated by a number of VOCs, including "thousands of gallons" of TCE observed fewer than 6 cases of cancer diagnosed between 1980 and 2001 in children aged 0−19 years, and did not exceed expected cases or types (ATSDR, 2006). A California community exposed to TCE in drinking water from contaminated wells was examined for cancer, with a specific emphasis on childhood cancer (<15 years old); however, the incidence did not exceed those expected for the community (Morgan and Cassady, 2002). A third study of

childhood cancer in relation to TCE in drinking water in Camp Lejeune, North Carolina is currently underway (GAO, 2007a, b).

4.10.1.1.3.3.2. *Childhood leukemia*. Childhood leukemia has been examined in relationship to TCE exposure (Cohn et al., 1994; Lagakos et al., 1986; Lowengart et al., 1987; McKinney et al., 1991; Costas et al., 2002; Shu et al., 1999). In a study examining drinking water exposure to TCE in 75 New Jersey towns, childhood leukemia, (including ALL) was significantly increased for girls (n = 6) diagnosed before age 20 years, but this was not observed for boys (Cohn et al., 1994). A community in Woburn, MA with contaminated well water including TCE experienced 20 cases of childhood leukemia, significantly more than expected (Lagakos et al., 1986). Further analysis by Costas et al. (2002) also observed a greater than 2-fold increase over expected cases of childhood leukemia. Cases were more likely to be male (76%), <9 years old at diagnosis (62%), breast-fed (OR: 10.17, 95% CI: 1.22−84.50), and exposed during pregnancy (adjusted OR: 8.33, 95% CI: 0.73−94.67). The highest risk was observed for exposure during pregnancy compared to preconception or postnatal exposure, and a dose-response was seen for exposure during pregnancy (Costas et al., 2002). In addition, family members of those diagnosed with childhood leukemia, including 13 siblings under age 19 at the time of exposure, had altered immune response, but an analysis looking at only these children was not done (Byers et al., 1988).

Case-control studies examined children diagnosed with ALL for parental occupational exposures and found a nonsignificant 2- to 4-fold increase of childhood leukemia risk for exposure to TCE during preconception, pregnancy, postnatally, or all developmental periods combined (Lowengart et al., 1987; McKinney et al., 1991; Shu et al., 1999). Some studies showed an elevated risk for maternal (Shu et al., 1999) or paternal exposure (Lowengart et al., 1987; McKinney et al., 1991), while others did not show an elevated risk for maternal (McKinney et al., 1991) or paternal exposure (Shu et al., 1999), possibly due to the small number of cases. No variability was observed in the developmental stages in Shu et al. (1999), although Lowengart et al. (1987) observed the highest risk to be paternal exposure to TCE after birth.

4.10.1.1.3.3.3. *CNS tumors*. In a case-control study of parental occupational exposures, paternal self-reported exposure to TCE was not significantly associated with neuroblastoma in the offspring (OR = 1.4, 95%CI: 0.7−2.9) (De Roos et al., 2001). Brain tumors have also been observed in the offspring of fathers exposed to TCE, but the odds ratio could not be determined (Peters et al., 1981, 1985).

4.10.1.1.3.3.4. *Age-dependent adjustment factors (ADAFs)*. According to U.S. EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic MOA. Therefore, because the weight of evidence supports a mutagenic MOA for TCE carcinogenicity in the kidney (see Section 4.4.7), and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the ADAFs should be applied, in accordance with the *Supplemental Guidance*.

4.10.1.2. *Later Lifestages*

Few studies examine the differential effects of TCE exposure for elderly adults (>65 years old). These limited studies suggest that older adults may experience increased adverse effects than younger adults. However, there is no further evidence for elderly individuals exposed to TCE beyond these studies.

Toxicokinetics in later lifestages are distinct from toxicokinetics in younger adults (Benedetti et al., 2007; Ginsberg et al., 2005). Studies have examined the age differences in TK after exposure to a mixture of six VOCs including TCE for humans (Mahle et al., 2007) and rats (Mahle et al., 2007; Rodriguez et al., 2007). In humans, the blood:air partition coefficient for adult males (20−82 years) was significantly ($p \leq 0.05$) higher (11.7 ± 1.9) compared to male (11.2 ± 1.8) or female (11.0 ± 1.6) children (3−10 years) (Mahle et al., 2007); when the data was stratified for adults above and below 55 years of age, there was no significant difference observed between adults (20−55 years) and aged (56−82) (data not reported). In rats, the difference in tissue:air partition coefficients also increased from PND10 to adult (2 months) to aged (2 years) rat (Mahle et al., 2007). TCE has also been measured in the brain of rats, with an increased level observed in older (2 year old) rats compared to adult (2 month old) rats (Rodriguez et al., 2007). It was also observed that aged rats reached steady state slower with higher concentrations compared to the adult rat; the authors suggest that the almost 2-fold greater percentage of body fat in the elderly is responsible for this response (Rodriguez et al., 2007). An age-related difference in CYP expression (Dorne et al., 2005), in particular CYP2E1 activity were observed in human liver (George et al., 1995), with the lowest activity in those >60 years and the highest in those <20 years old (Parkinson et al., 2004). Also, GST expression has been observed to decrease with age in human lymphocytes, with the lowest expression in those aged 60−80 years old (van Lieshout and Peters, 1998).

One cohort of TCE exposed metal degreasers found an increase in psychoorganic syndrome and increased vibration threshold related to increasing age (Rasmussen et al., 1993a, b, c), although the age groups were ≤29 years, 30−39 years, and 40+ years, but the age ranged only from 18−68 years and did not examine >65 years as a separate category.

4.10.2. Other Susceptibility Factors

Aside from age, many other factors may affect susceptibility to TCE toxicity. A partial list of these factors includes gender, genetic polymorphisms, pre-existing disease status, nutritional status, diet, and previous or concurrent exposures to other chemicals. The toxicity that results due to changes in multiple factors may be quite variable, depending on the exposed population and the type of exposure. Qualitatively, the presence of multiple susceptibility factors will increase the variability that is seen in a population response to TCE toxicity.

4.10.2.1. *Gender*

Individuals of different genders are physiologically, anatomically, and biochemically different. Males and females can differ greatly in many physiological parameters such as body composition, organ function, and ventilation rate, which can influence the toxicokinetics of chemicals and their metabolites in the body (Gandhi et al., 2004; Gochfeld, 2007).

4.10.2.1.1. ***Gender-specific toxicokinetics.*** Chapter 3 describes the toxicokinetics of TCE. Gender-specific information is described below for absorption, distribution, metabolism, and excretion, followed by available gender-specific PBPK models.

4.10.2.1.1.1. ***Absorption.*** As discussed in Section 3.1, exposure to TCE may occur via inhalation, ingestion, and skin absorption. Exposure via inhalation is proportional to the ventilation rate, duration of exposure, and concentration of expired air, and women have increased ventilation rates during exercise compared to men (Gochfeld, 2007). Percent body fat varies with gender (Gochfeld, 2007), which for lipophilic compounds such as TCE will affect absorption and retention of the absorbed dose. After experimental exposure to TCE, women were found to absorb a lower dose due to lower alveolar intake rates compared to men (Sato, 1993; Sato et al., 1991b).

4.10.2.1.1.2. ***Distribution.*** Both human and animal studies provide clear evidence that TCE distributes widely to all tissues of the body (see Section 3.2). The distribution of TCE to specific organs will depend on organ blood flow and the lipid and water content of the organ, which may vary between genders (Gochfeld, 2007). After experimental exposure to humans, higher distribution of TCE into fat tissue was observed in women leading to a greater blood concentration 16 hours after exposure compared to men (Sato, 1993; Sato et al., 1991b). In experimental animals, male rats generally have higher levels of TCE in tissues compared to female rats, likely due to gender differences in metabolism (Lash et al., 2006). In addition, TCE

has been observed in the male reproductive organs (epididymis, vas deferens, testis, prostate, and seminal vesicle) (Zenick et al., 1984).

4.10.2.1.1.3. *Metabolism*. Section 3.3 describes the metabolic processes involved in the metabolism of TCE, including CYP and GST enzymes. In addition, the role of metabolism in male reproductive toxicity is discussed in Section 4.8.1.3.2. In general, there is some indication that TCE metabolism is different between males and females, with females more rapidly metabolizing TCE after oral exposure to rats (Lash et al., 2006), intraperitoneal injections in rats (Verma and Rana, 2003), and in mouse, rat and human liver microsomes (Elfarra et al., 1998).

CYP expression may differ between genders (Gandhi et al., 2004; Gochfeld, 2007; Lash et al., 2006; Parkinson et al., 2004). CYP2E1 was detected in the epididymis and testes of mice (Forkert et al., 2002), and CYP2E1 and GST-α has been detected in the ovaries of rats (Wu and Berger, 2008), indicating that metabolism of TCE can occur in both the male and female reproductive tracts. Unrelated to TCE exposure, there is no gender-related difference in CYP2E1 activity observed in human liver microsomes (Parkinson et al., 2004). One study of TCE exposure in mice observed induced CYP2E1 expression in the liver of males only (Nakajima et al., 2000). Male rats have been shown to have higher levels of TCE metabolites in the liver (Lash et al., 2006), and lower levels of TCE metabolites in the kidney (Lash et al., 2006) compared to female rats. However, another study did not observe ant sex-related differences in the metabolism of TCE in rats (Nakajima et al., 1992a).

Unlike CYP-mediated oxidation, quantitative differences in the polymorphic distribution or activity levels of GST isoforms in humans are not presently known. However, the available data (Lash et al., 1999a, b) do suggest that significant variation in GST-mediated conjugation of TCE exists in humans. One study observed that GSH conjugation is higher in male rats compared to female rats (Lash et al., 2000); however, it has also been speculated that any gender difference may be due to a polymorphism in GSH conjugation of TCE rather than a true gender difference (Lash et al., 1999a). Also, induction of PPARα expression in male mice was greater than that in females (Nakajima et al., 2000).

4.10.2.1.1.4. *Excretion*. The major processes of excretion of TCE and its metabolites are discussed in Section 3.4. Two human voluntary inhalation exposure studies observed the levels of TCE and its metabolites in exhaled breath and urine (Kimmerle and Eben, 1973; Nomiyama and Nomiyama, 1971). Increased levels of TCE in exhaled breath in males were observed in one human voluntary inhalation exposure study of 250−380 ppm for 160 minutes (Nomiyama and Nomiyama, 1971), but no difference was observed in another study of 40 ppm for 4 hours or 50 ppm for 4 hours for 5 days (Kimmerle and Eben, 1973).

After experimental exposure to TCE, women were generally found to excrete higher levels of TCE and TCA compared to men (Kimmerle and Eben, 1973; Nomiyama and Nomiyama, 1971). However, other studies observed an increase in TCE in the urine of males (Inoue et al., 1989), an increase in TCA in the urine of males (Sato et al., 1991b), or no statistically significant ($p > 0.10$) gender difference for TCA in the urine (Inoue et al., 1989). Others found that the urinary elimination half-life of TCE metabolites is longer in women compared to men (Ikeda, 1977; Ikeda and Imamura, 1973).

In addition to excretion pathways that occur in both genders, excretion occurs uniquely in men and women. In both humans and experimental animals, it has been observed that females can excrete TCE and metabolites in breast milk (Fisher et al., 1990, 1997; Hamada and Tanaka, 1995; Pellizzari et al., 1982), while males can excrete TCE and metabolites in seminal fluid (Forkert et al., 2003; Zenick et al., 1984).

4.10.2.1.1.5. ***Physiologically-based pharmacokinetic (PBPK) models.*** Gender-specific differences in uptake and metabolism of TCE were incorporated into a PBPK model using human exposure data (Fisher et al., 1998). The chemical-specific parameters included cardiac output at rest, ventilation rates, tissue volumes, blood flow, and fat volume. This model found that gender differences for the toxicokinetics of TCE are minor.

4.10.2.1.2. ***Gender -specific effects.***

4.10.2.1.2.1. ***Gender susceptibility to noncancer outcomes.***

4.10.2.1.2.1.1. *Liver toxicity*. No gender susceptibility to noncancerous outcomes in the liver was observed. A detailed discussion of the studies examining the effects of TCE on the liver can be found in Section 4.4.

4.10.2.1.2.1.2. *Kidney toxicity*. A detailed discussion of the studies examining the noncancer effects of TCE on the kidney can be found in Section 4.5. A residential study found that females aged 55−64 years old had an elevated risk of kidney disease (RR = 4.57, 99% CI: 2.10−9.93), although an elevated risk of urinary tract disorders was reported for both males and females (Burg et al., 1995). Additionally, a higher rate of diabetes in females exposed to TCE was reported in two studies (Burg et al., 1995; Davis et al., 2005). In rodents, however, and kidney weights were increased more in male mice than in females (Kjellstrand et al., 1983a, b), and male rats have exhibited increased renal toxicity to TCE (Lash et al., 1998, 2001).

4.10.2.1.2.1.3. *Immunotoxicity*. A detailed discussion of the studies examining the immunotoxic effects of TCE can be found in Section 4.6. Most of the immunotoxicity studies present data stratified by sex. The prevalence of exposure to TCE is generally lower in women compared with men. In men, the studies generally reported odds ratios between 2.0 and 8.0, and in women, the odds ratios were between 1.0 and 2.0. Based on small numbers of cases, an occupational study of TCE exposure found an increased risk for systemic sclerosis for men (OR: 4.75, 95% CI: 0.99−21.89) compared to women (OR: 2.10; 95% CI: 0.65−6.75) (Diot et al., 2002). Another study found similar results, with an elevated risk for men with a maximum intensity, cumulative intensity and maximum probability of exposure to TCE compared to women (Nietert et al., 1998). These two studies, along with one focused exclusively on the risk of scleroderma to women (Garabrant et al., 2003), were included in a meta-analysis conducted by the U.S. EPA resulting in a combined estimate for "any" exposure, was OR = 2.5 (95% CI: 1.1, 5.4) for men and OR = 1.2 (95% CI: 0.58, 2.6) in women.

4.10.2.1.2.1.4. *Respiratory toxicity*. No gender susceptibility to noncancerous outcomes in the respiratory tract was observed. A detailed discussion of the studies examining the respiratory effects of TCE can be found in Section 4.7.

4.10.2.1.2.1.5. *Reproductive toxicity*. A detailed discussion of the studies examining the gender-specific noncancer reproductive effects of TCE can be found in Section 4.8.1.

Studies examining males after exposure to TCE observed altered sperm morphology and hyperzoospermia (Chia et al., 1996), altered endocrine function (Chia et al., 1997; Goh et al., 1998), decreased sexual drive and function (Bardodej and Vyskocil, 1956; El Ghawabi et al., 1973; Saihan et al., 1978), and altered fertility to TCE exposure. Infertility was not associated with TCE exposure in other studies (Forkert et al., 2003; Sallmén et al., 1998), and sperm abnormalities were not observed in another study (Rasmussen et al., 1988).

There is more limited evidence for reproductive toxicity in females. There are epidemiological indicators of a possible effect of TCE exposure on female fertility (Sallmén et al., 1995), increased rate of miscarriage (ATSDR, 2001), and menstrual cycle disturbance (ATSDR, 2001; Bardodej and Vyskocil, 1956; Zielinski, 1973). In experimental animals, the effects on female reproduction include evidence of reduced *in vitro* oocyte fertilizability in rats (Berger and Horner, 2003; Wu and Berger, 2007, 2008). However, in other studies that assessed reproductive outcome in female rodents (Cosby and Dukelow, 1992; George et al., 1985, 1986; Manson et al., 1984), there was no evidence of adverse effects of TCE exposure on female reproductive function.

4.10.2.1.2.1.6. *Developmental toxicity*. A detailed discussion of the studies examining the gender-specific noncancer developmental effects of TCE can be found in Section 4.8.3. Only one study of contaminated drinking water exposure in Camp Lejeune, North Carolina observed a higher risk of SGA in males (ATSDR, 1998; Sonnenfeld et al., 2001).

4.10.2.1.2.2. *Gender susceptibility to cancer outcomes*. A detailed discussion of the studies examining the carcinogenic effects of TCE can be found on the liver in Section 4.4, on the kidney in Section 4.5, in the immune system in Section 4.6.4, in the respiratory system in Sections 4.7.1.2 and 4.7.3, and on the reproductive system in Section 4.8.2.

4.10.2.1.2.2.1. *Liver cancer*. An elevated risk of liver cancer was observed for females in both human (Raaschou-Nielsen et al., 2003) and rodent (Elfarra et al., 1998) studies. In addition, gallbladder cancer was significantly elevated for women (Raaschou-Nielsen et al., 2003). A detailed discussion of the studies examining the gender-specific liver cancer effects of TCE can be found in Section 4.4.

4.10.2.1.2.2.2. *Kidney cancer*. One study of occupational exposure to TCE observed an increase in renal cell carcinoma for women compared to men (Dosemeci et al., 1999), but no gender difference was observed in other studies (Pesch et al., 2000; Raaschou-Nielsen et al., 2003). Blair et al. (1998) 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. Exposure differences between males and females in Dosemeci et al. (1999) may explain their finding. These studies, however, provide little information to evaluate susceptibility between sexes because of their lack of quantitative exposure assessment and lower statistical power. A detailed discussion of the studies examining the gender-specific kidney cancer effects of TCE can be found in Section 4.5.

4.10.2.1.2.2.3. *Cancers of the immune system*. Two drinking water studies suggest that there may be an increase of leukemia (Cohn et al., 1994; Fagliano et al., 1990) and NHL (Cohn et al., 1994) among females. An occupational study also observed an elevated risk of leukemia in females (Raaschou-Nielsen et al., 2003), although study of contaminated drinking water in Woburn, Massachusetts observed an increased risk of childhood leukemia in males (Costas et al., 2002). A detailed discussion of the studies examining the gender-specific cancers of the immune system following TCE exposure can be found in Section 4.6.4.

4.10.2.1.2.2.4. *Respiratory cancers*. One study observed significantly elevated risk of lung cancer following occupational TCE exposure for both men and women, although the risk was found to be higher for women (Raaschou-Nielsen et al., 2003). This same study observed a nonsignificant elevated risk in both men and women for laryngeal cancer, again with an increased risk for women (Raaschou-Nielsen et al., 2003). Conversely, a study of Iowa residents with TCE-contaminated drinking water observed a 7-fold increased annual age-adjusted incidence for males compared to females (Isacson et al., 1985). However, other studies did not observe a gender-related difference (ATSDR, 2003a; Blair et al., 1998; Hansen et al., 2001). A detailed discussion of the studies examining the gender-specific respiratory cancers following TCE exposure can be found in Sections 4.7.1.2 and 4.7.3.

4.10.2.1.2.2.5. *Reproductive cancers*. Breast cancer in females and prostate cancer in males was reported after exposure to TCE in drinking water (Isacson et al., 1985). A statistically elevated risk for cervical cancer, but not breast, ovarian or uterine cancer, was observed in women in another study (Raaschou-Nielsen et al., 2003). This study also did not observe elevated prostate or testicular cancer (Raaschou-Nielsen et al., 2003). A detailed discussion of the studies examining the gender-specific reproductive cancers following TCE exposure can be found in Section 4.8.2.

4.10.2.1.2.2.6. *Other Cancers*. Bladder and rectal cancer was increased in men compared to women after exposure to TCE in drinking water, but no gender difference was observed for colon cancer (Isacson et al., 1985). After occupational TCE exposure, bladder, stomach, colon, and esophageal cancer was nonsignificantly elevated in women compared to men (Raaschou-Nielsen et al., 2003).

4.10.2.2. *Genetic Variability*

Section 3.3 describes the metabolic processes involved in the metabolism of TCE. Human variation in response to TCE exposure may be associated with genetic variation. TCE is metabolized by both CYP and GST; therefore, it is likely that polymorphisms will alter the response to exposure (Garte et al., 2001; Nakajima and Aoyama, 2000), as well as other chemicals that may alter the metabolism of TCE (Lash et al., 2007). It is important to note that even with a given genetic polymorphism, metabolic expression is not static, and depends on lifestage (see Section 4.10.1.1.2), obesity (see Section 4.10.2.4.1), and alcohol intake (see Section 4.10.2.5.1).

4.10.2.2.1. ***CYP genotypes.*** Variability in CYP expression occurs both within humans (Dorne et al., 2005) and across experimental animal species (Nakajima et al., 1993). In particular, increased CYP2E1 activity may lead to increased susceptibility to TCE (Lipscomb et al., 1997). The CYP2E1*3 allele and the CYP2E1*4 allele were more common among those who developed scleroderma who were exposed to solvents including TCE (Povey et al., 2001). A PBPK model of CYP2E1 expression after TCE exposure has been developed for rats and humans (Yoon et al., 2007).

In experimental animals, toxicokinetics of TCE differed among CYP2E1 knockout and wild-type mice (Kim and Ghanayem, 2006). This study found that exhalation was more prevalent among the knockout mice, whereas urinary excretion was more prevalent among the wild-type mice. In addition, the dose was found to be retained to a greater degree by the knockout mice compared to the wild-type mice.

4.10.2.2.2. ***GST genotype.*** There is a possibility that GST polymorphisms could play a role in variability in toxic response (Caldwell and Keshava, 2006), but this has not been sufficiently tested (NRC, 2006). One study of renal cell cancer in workers exposed to TCE demonstrated a significant increased for those with GSTM1+ and GSTT1+ polymorphisms, compared to a negative risk for those with GSTM1- and GSTT1-polymorphisms (Brüning et al., 1997). However, another study did not confirm this hypothesis, observing no clear relationship between GSTM1 and GSTT1 polymorphisms and renal cell carcinoma among TCE exposed individuals, although they did see a possible association with the homozygous wild-type allele GSTP1*A (Wiesenhütter et al., 2007). A third study unrelated to TCE exposure found GSTT1- to be associated with an increased risk of renal cell carcinoma, but no difference was seen for GSTM1 and GSTP1 alleles (Sweeney et al., 2000).

4.10.2.2.3. ***Other genotypes.*** Other genetic polymorphisms could play a role in variability in toxic response, in particular TCE-related skin disorders. Studies have found that many TCE-exposed patients diagnosed with skin conditions exhibited the slow-acetylator NAT2 genotype (Huang et al., 2002; Nakajima et al., 2003); whereas there was no difference in NAT2 status for those diagnosed with renal cell carcinoma (Wiesenhütter et al., 2007). Other studies have found that many TCE-exposed patients diagnosed with skin conditions expressed variant HLA alleles (Li et al., 2007; Yue et al., 2007), in particular HLA-B*1301 which is more common in Asians compared to whites (Cao et al., 2001; Williams et al., 2001); or TNF α-308 allele (Dai et al., 2004). Also, an *in vitro* study of human lung adenocarcinoma cells exposed to TCE varied in response based on their p53 status, with p53-wild-type cells resulting in severe cellular damage, but not the p53-null cells (Chen et al., 2002).

4.10.2.3. *Race/Ethnicity*

Different racial or ethnic groups may express metabolic enzymes in different ratios and proportions due to genetic variability (Garte et al., 2001). In particular, ethnic variability in CYP expression has been reported (Dorne et al., 2005; McCarver et al., 1998; Parkinson et al., 2004; Shimada et al., 1994; Stephens et al., 1994). It has been observed that the metabolic rate for TCE may differ between the Japanese and Chinese (Inoue et al., 1989). Also, body size varies among ethnic groups, and increased body size was related to increased absorption of TCE and urinary excretion of TCE metabolites (Sato et al., 1991b).

4.10.2.4. *Pre-Existing Health Status*

It is known that kidney and liver diseases can affect the clearance of chemicals from the body, and therefore, poor health may lead to increased half-lives for TCE and its metabolites. There is some data indicating that obesity/metabolic syndrome, diabetes and hypertension may increase susceptibility to TCE exposure through altered toxicokinetics. In addition, some of these conditions lead to increased risk for adverse effects that have also been associated with TCE exposure, though the possible interaction between TCE and known risk factors for these effects is not understood.

4.10.2.4.1. ***Obesity and metabolic syndrome.*** TCE is lipophilic and stored in adipose tissue; therefore, obese individuals may have an increased body burden of TCE (Clewell et al., 2000). Immediately after exposure, blood concentrations are higher and urinary excretion of metabolites are faster in thin men than obese men due to the storage of TCE in the fat. However, the release of TCE from the fat tissue beginning three hours after exposure reverses this trend and obese men have increased blood concentrations and urinary excretion of metabolites are compared to thin men (Sato, 1993; Sato et al., 1991b). This study also reported that increased body size was related to increased absorption and urinary excretion of TCE metabolites (Sato et al., 1991b). After evaluating the relationship between mean daily uptake and mean minute volume, body weight, lean body mass, and amount of adipose tissue, the variation in uptake was more closely correlated with lean body mass, but not adipose tissue content (Monster et al., 1979). Thus, adipose tissue may play an important role in postexposure distribution, but is not a primary determinant of TCE uptake. Increased CYP2E1 expression has been observed in obese individuals (McCarver et al., 1998). Accumulation into adipose tissue may prolong internal exposures (Davidson and Beliles, 1991; Lash et al., 2000), as evidenced by increased durations of elimination in subjects with larger body mass indices (Monster, 1979).

In addition, individuals with high BMI are at increased risk of some of the same health effects associated with TCE exposure. For example, renal cell carcinoma, liver cancer, and

prostate cancer may be positively associated with BMI or obesity (Asal et al., 1988a, b; Benichou et al., 1998; El-Serag and Rudolph, 2007; Wigle et al., 2008). However, whether and how TCE interacts with known risk factors for such diseases is unknown, as existing epidemiologic studies have only examined these factors as possible confounders for effects associated with TCE, or vice versa (Charbotel et al., 2006; Krishnadasan et al., 2008).

4.10.2.4.2. ***Diabetes.*** A higher rate of diabetes in females exposed to TCE was reported in two studies (Burg et al., 1995; Davis et al., 2005). Whether the TCE may have caused the diabetes or the diabetes may have increased susceptibility to TCE is not clear. However, it has been observed that CYP2E1 expression is increased in obese Type II diabetics (Wang et al., 2003), and in poorly controlled Type I diabetics (Song et al., 1990), which may consequently alter the metabolism of TCE.

4.10.2.4.3. ***Hypertension.*** One study found no difference in risk for renal cell carcinoma among those diagnosed with hypertension among those living in an area with high TCE exposure; however, a slightly elevated risk was seen for those being treated for hypertension (OR: 1.57, 95% CI: 0.90−2.72) (Charbotel et al., 2006). Unrelated to TCE exposure, hypertension has been associated with increase risk of renal cell carcinoma in women (Benichou et al., 1998).

4.10.2.5. ***Lifestyle Factors and Nutrition Status***

4.10.2.5.1. ***Alcohol intake.*** A number of studies have examined the interaction between TCE and ethanol exposure in both humans (Bardodej and Vyskocil, 1956; Barret et al., 1984; McCarver et al., 1998; Müller et al., 1975; Sato, 1993; Sato et al., 1981, 1991a; Stewart et al., 1974) and experimental animals (Kaneko et al., 1994; Larson and Bull, 1989; Nakajima et al., 1988, 1990, 1992b; Okino et al., 1991; Sato et al., 1980, 1983; Sato and Nakajima, 1985; White and Carlson, 1981).

The coexposure causes metabolic inhibition of TCE in humans (Müller et al., 1975; Windemuller and Ettema, 1978), male rats (Kaneko et al., 1994; Larson and Bull, 1989; Nakajima et al., 1988, 1990; Nakanishi et al., 1978; Okino et al., 1991; Sato and Nakajima, 1985; Sato et al., 1981), and rabbits (White and Carlson, 1981). Similarly, individuals exposed to TCE reported an increase in alcohol intolerance (Bardodej and Vyskocil, 1956; Grandjean et al., 1955; Rasmussen and Sabroe, 1986). Disulfiram, used to treat alcoholism, has also been found to decrease the elimination of TCE and TCA (Bartonicek and Teisinger, 1962).

A "degreasers flush" has been described, reflecting a reddening of the face of those working with TCE after drinking alcohol, and measured an elevated level of TCE in exhaled breath compared to nondrinkers exposed to TCE (Stewart et al., 1974). This may be due to

increased CYP2E1 expression in those that consume alcohol (Caldwell et al., 2008; Liangpunsakul et al., 2005; Lieber, 2004; McCarver et al., 1998; Parkinson et al., 2004; Perrot et al., 1989), which has also been observed in male rats fed alcohol (Nakajima et al., 1992b), although another study of male rats observed that ethanol did not decrease CYP activity (Okino et al., 1991). It is important to note that there a further increased response of TCE and ethanol has been reported when also combined with low fat diets or low carbohydrate diets in male rats (Sato et al., 1983).

Since the liver is a target organ for both TCE and alcohol, decreased metabolism of TCE could be related to cirrhosis of the liver as a result of alcohol abuse (McCarver et al., 1998), and an in increase in clinical liver impairment along with degreasers flush has been observed (Barret et al., 1984).

The central nervous system may also be impacted by the coexposure. Individuals exposed to TCE and ethanol reported an increase in altered mood states (Reif et al., 2003), decreased mental capacity as described as small increases in functional load (Windemuller and Ettema, 1978), and those exposed to TCE and tetrachloroethylene who consumed alcohol had an elevated color confusion index (Valic et al., 1997).

4.10.2.5.2. ***Tobacco smoking.*** Individuals who smoke tobacco may be at increased risk of the health effects from TCE exposure. One study examining those living in an area with high TCE exposure found an increasing trend of risk ($p = 0.008$) for renal cell carcinoma among smokers, with the highest OR among those with ≥40 pack-years (OR = 3.27, 95% CI: 1.48−7.19) (Charbotel et al., 2006). It has been shown that renal cell carcinoma is independently associated with smoking in a dose-response manner (Yuan et al., 1998), particularly in men (Benichou et al., 1998).

A number of factors correlated to smoking (e.g., socioeconomic status, diet, alcohol consumption) may positively confound results if greater smoking rates were over-represented in a cohort (Raaschou-Nielsen et al., 2003). Absence of smoking information, on the other hand, could introduce a negative bias. Morgan and Cassidy (2002) noted the relatively high education high income levels, and high access to health care of subjects in this study compared to the averages for the county as a whole likely leads to a lower smoking rate. Garabrant et al. (1988) similarly attributed their observations to negative selection bias introduced when comparison is made to national mortality rats known as "the healthy worker effect."

4.10.2.5.3. ***Nutritional status.*** Malnutrition may also increase susceptibility to TCE. Bioavailability of TCE after oral and intravenous exposure increased with fasting from approximately 63% in nonfasted rats to greater than 90% in fasted rats, with blood levels in

fasted rats were elevated 2−3-fold, and increased half-life in the blood of fasted rats (D'Souza et al., 1985). Food deprivation (Sato and Nakajima, 1985) and carbohydrate restriction (Nakajima et al., 1982; Sato and Nakajima, 1985) enhanced metabolism of TCE in male rats, but this was not observed for dietary changes in protein or fat levels (Nakajima et al., 1982).

Vitamin intake may also alter susceptibility to TCE. An *in vitro* study of cultured normal human epidermal keratinocyte demonstrated an increased lipid peroxidation in a dose-dependant manner after exposure to TCE, which were then attenuated by exposure to Vitamin E (Ding et al., 2006).

4.10.2.5.4. ***Physical activity.*** Increased inhalation during physical activity leads increases TCE concentrations in the alveoli when compared to inhalation in a resting state (Astrand, 1975). Studies have examined the time course of inhaled TCE and metabolites in blood and urine in individuals with different workloads (Astrand and Ovrum, 1976; Jakubowski and Wieczorek, 1988; Monster et al., 1976; Vesterberg et al., 1976; Vesterberg and Astrand, 1976). These studies demonstrate that an increase in pulmonary ventilation increases the amount of TCE taken up during exposure (Astrand and Ovrum, 1976; Jakubowski and Wieczorek, 1988; Monster et al., 1976; Sato, 1993).

The Rocketdyne aerospace cohort exposed to TCE (and other chemicals) found a protective effect with high physical activity, but only after controlling for TCE exposure and socioeconomic status (OR = 0.55, 95% CI: 0.32−0.95, *p* trend = 0.04) (Krishnadasan et al., 2008). In general, physical activity may provide a protective effect for prostate cancer (Wigle et al., 2008) (see Section 4.8.3.1.1).

4.10.2.5.5. ***Socioeconomic status.*** Socioeconomic status (SES) can be an indicator for a number of coexposures, such as increased tobacco smoking, poor diet, education, income, and health care access, which may play a role in the results observed in the health effects of TCE exposure (Morgan and Cassidy, 2002).

Children's exposure to TCE was measured in a low SES community, as characterized by income, educational level, and receipt of free or reduced cost school meals (Sexton et al., 2005); however, this study did not compare data to a higher SES community, nor examine health effects.

An elevated risk of NHL and esophagus/adenocarcinoma after exposure to TCE was observed for blue-collar workers compared to white collar and unknown SES (Raaschou-Nielsen et al., 2003). Authors speculate that these results could be confounding due to other related factors to SES such as smoking.

4.10.3. Uncertainty of Database for Susceptible Populations

There is some evidence that certain subpopulations may be more susceptible to exposure to TCE. These subpopulations include early and later lifestages, gender, genetic polymorphisms, race/ethnicity, pre-existing health status, and lifestyle factors and nutrition status. Although there is more information on early life exposure to TCE than on other potentially susceptible populations, there remain a number of uncertainties regarding children's susceptibility. Improved PBPK modeling for using childhood parameters early lifestages as recommended by the NRC (2006), and validation of these models, will aid in determining how variations in metabolic enzymes affect TCE metabolism. In particular, the NRC states that it is prudent to assume children need greater protection than adults—unless sufficient data are available to justify otherwise (NRC, 2006).

More studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential life stage-related TCE toxicity. Because the neurological effects of TCE constitute the most sensitive endpoints of concern for noncancer effects, it is quite likely that the early lifestages may be more susceptible to these outcomes than are adults. Lifestage-specific neurotoxic effects, particularly in the developing fetus, need further evaluation. It is important to consider the use of age-appropriate testing for assessment of these and other outcomes, both for cancer and noncancer outcomes. Data specific to the carcinogenic effects of TCE exposure during the critical periods of development of experimental animals and humans also are sparse.

There is a need to better characterize the implications of TCE exposures to susceptible populations. There is suggestive evidence that there may be greater susceptibility for exposures to the elderly. Gender and race/ethnic differences in susceptibility are likely due to variation in physiology and exposure, and genetic variation likely has an effect on the toxicokinetics of TCE. Diminished health status (e.g., impaired kidney liver or kidney), alcohol consumption, tobacco smoking, and nutritional status will likely affect an individual's ability to metabolize TCE. In addition, further evaluation of the effects due to coexposures to other compounds with similar or different MOAs need to be evaluated. Future research should better characterize possible susceptibility for certain lifestages or populations.

4.11. HAZARD CHARACTERIZATION

4.11.1. Characterization of Noncancer Effects

4.11.1.1. *Neurotoxicity*

Both human and animal studies have associated TCE exposure with effects on several neurological domains. The strongest neurological evidence of hazard in humans is for changes

in trigeminal nerve function or morphology and impairment of vestibular function. Fewer and more limited evidence exists in humans on delayed motor function, and changes in auditory, visual, and cognitive function or performance. Acute and subchronic animal studies show morphological changes in the trigeminal nerve, disruption of the peripheral auditory system leading to permanent function impairments and histopathology, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Additional acute studies reported structural or functional changes in hippocampus, such as decreased myelination or decreased excitability of hippocampal CA1 neurons, although the relationship of these effects to overall cognitive function is not established. Some evidence exists for motor-related changes in rats/mice exposed acutely/subchronically to TCE, but these effects have not been reported consistently across all studies.

Epidemiologic evidence supports a relationship between TCE exposure and trigeminal nerve function changes, with multiple studies in different populations reporting abnormalities in trigeminal nerve function in association with TCE exposure (Barret et al., 1982, 1984, 1987; Feldman et al., 1988, 1992; Kilburn and Warshaw, 1993; Ruitjen et al., 2001; Kilburn, 2002a; Mhiri et al., 2004). Of these, two well conducted occupational cohort studies, each including more than 100 TCE-exposed workers without apparent confounding from multiple solvent exposures, additionally reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA (Barret et al., 1984; Barret et al., 1987). Limited additional support is provided by a positive relationship between prevalence of abnormal trigeminal nerve or sensory function and cumulative exposure to TCE (most subjects) or CFC-113 (<25% of subjects) (Rasmussen et al., 1993c). Test for linear trend in this study was not statistically significant and may reflect exposure misclassification since some subjects included in this study did not have TCE exposure. The lack of association between TCE exposure and overall nerve function in three small studies (trigeminal: El-Ghawabi et al., 1973; ulnar and medial: Triebig et al., 1982, 1983) does not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment because of limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. Laboratory animal studies have also shown TCE-induced changes in the trigeminal nerve. Although one study reported no significant changes in trigeminal somatosensory evoked potential in rats exposed to TCE for 13 weeks (Albee et al., 2006), there is evidence of morphological changes in the trigeminal nerve following short-term exposures in rats (Barret et al., 1991, 1992).

Human chamber, occupational, geographic based/drinking water, and laboratory animal studies clearly established TCE exposure causes transient impairment of vestibular function. Subjective symptoms such as headaches, dizziness, and nausea resulting from occupational

(Granjean et al., 1955; Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith et al., 1970), environmental (Hirsch et al., 1996), or chamber exposures (Stewart et al., 1970; Smith et al., 1970) have been reported extensively. A few laboratory animal studies have investigated vestibular function, either by promoting nystagmus or by evaluating balance (Niklasson et al., 1993; Tham et al., 1979; Tham et al., 1984; Umezu et al., 1997).

In addition, mood disturbances have been reported in a number of studies, although these effects also tend to be subjective and difficult to quantify (Gash et al., 2007; Kilburn and Warshaw, 1993; Kilburn, 2002a, 2002b; McCunney et al., 1988; Mitchell et al., 1969; Rasmussen and Sabroe, 1986; Troster and Ruff, 1990), and a few studies have reported no effects from TCE on mood (Reif et al., 2003; Triebig et al., 1976, 1977a). Few comparable mood studies are available in laboratory animals, although both Moser et al. (2003) and Albee et al. (2006) report increases in handling reactivity among rats exposed to TCE. Finally, significantly increased number of sleep hours was reported by Arito et al. (1994) in rats exposed via inhalation to 50–300-ppm TCE for 8 hours/day for 6 weeks.

Four epidemiologic studies of chronic exposure to TCE observed disruption of auditory function. One large occupational cohort study showed a statistically significant difference in auditory function with cumulative exposure to TCE or CFC-113 as compared to control groups after adjustment for possible confounders, as well as a positive relationship between auditory function and increasing cumulative exposure (Rasmussen et al., 1993b). Of the three studies based on populations from ATSDR's TCE Subregistry from the National Exposure Registry, more limited than Rasmussen et al. (1993b) due to inferior exposure assessment, Burg et al. (1995) and Burg and Gist (1999) reported a higher prevalence of self-reported hearing impairments. The third study reported that auditory screening revealed abnormal middle ear function in children less than 10 years of age, although a dose-response relationship could not be established and other tests did not reveal differences in auditory function (ATSDR, 2003a). Further evidence for these effects is provided by numerous laboratory animal studies demonstrating that high dose subacute and subchronic TCE exposure in rats disrupts the auditory system leading to permanent functional impairments and histopathology.

Studies in humans exposed under a variety of conditions, both acutely and chronically, report impaired visual functions such as color discrimination, visuospatial learning tasks, and visual depth perception in subjects with TCE exposure. Abnormalities in visual depth perception were observed with a high acute exposure to TCE under controlled conditions (Vernon and Ferguson, 1969). Studies of lower TCE exposure concentrations also observed visuofunction effects. One occupational study (Rasmussen et al., 1993b) reported a statistically significant positive relationship between cumulative exposure to TCE or CFC-113 and visual gestalts learning and retention among Danish degreasers. Two studies of populations living in a

community with drinking water containing TCE and other solvents furthermore suggested changes in visual function (Kilburn et al., 2002a; Reif et al., 2003). These studies used more direct measures of visual function as compared to Rasmussen et al. (1993b), but their exposure assessment is more limited because TCE exposure is not assigned to individual subjects (Kilburn et al., 2002a), or because there are questions regarding control selection (Kilburn et al., 2002a) and exposure to several solvents (Kilburn et al., 2002a; Reif et al., 2003).

Additional evidence of effects of TCE exposure on visual function is provided by a number of laboratory animal studies demonstrating that acute or subchronic TCE exposure causes changes in visual evoked responses to patterns or flash stimulus (Boyes et al., 2003, 2005; Blain et al., 1994). Animal studies have also reported that the degree of some effects is correlated with simultaneous brain TCE concentrations (Boyes et al., 2003, 2005) and that, after a recovery period, visual effects return to control levels (Blain et al., 1994; Rebert et al., 1991). Overall, the human and laboratory animal data together suggest that TCE exposure can cause impairment of visual function, and some animal studies suggest that some of these effects may be reversible with termination of exposure.

Studies of human subjects exposed to TCE either acutely in chamber studies or chronically in occupational settings have observed deficits in cognition. Five chamber studies reported statistically significant deficits in cognitive performance measures or outcome measures suggestive of cognitive effects (Stewart et al., 1970; Gamberale et al., 1976; Triebig et al., 1976, 1977a; Gamberale et al., 1977). Danish degreasers with high cumulative exposure to TCE or CFC-113 had a high risk (OR = 13.7, 95% CI: 2.0–92.0) for psychoorganic syndrome characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative compared to workers with low cumulative exposure. Studies of populations living in a community with contaminated groundwater also reported cognitive impairments (Kilburn and Warshaw, 1993; Kilburn, 2002a), although these studies carry less weight in the analysis because TCE exposure is not assigned to individual subjects and their methodological design is weaker.

Laboratory studies provide some additional evidence for the potential for TCE to affect cognition, although the predominant effect reported has been changes in the time needed to complete a task, rather than impairment of actual learning and memory function (Kulig et al., 1987; Kishi et al., 1993; Umezu et al., 1997). In addition, in laboratory animals, it can be difficult to distinguish cognitive changes from motor-related changes. However, several studies have reported structural or functional changes in the hippocampus, such as decreased myelination (Issacson et al., 1990; Isaacson and Taylor, 1989) or decreased excitability of hippocampal CA1 neurons (Ohta et al., 2001), although the relationship of these effects to overall cognitive function is not established.

Two studies of TCE exposure, one chamber study of acute exposure duration and one occupational study of chronic duration, reported changes in psychomotor responses. The chamber study of Gamberale et al. (1976) reported a dose-related decrease in performance in a choice reaction time test in healthy volunteers exposed to 100 and 200-ppm TCE for 70 minutes as compared to the same subjects without exposure. Rasmussen et al. (1993c) reported a statistically significant association with cumulative exposure to TCE or CFC-113 and dyscoordination trend among Danish degreasers. Observations in a third study (Gun et al., 1978) are difficult to judge given the author's lack of statistical treatment of data. In addition, Gash et al. (2007) reported that 14 out of 30 TCE-exposed workers exhibited significantly slower fine motor hand movements as measured through a movement analysis panel test. Studies of population living in communities with TCE and other solvents detected in groundwater supplies reported significant delays in simple and choice reaction times in individuals exposed to TCE in contaminated groundwater as compared to referent groups (Kilburn, 2002a; Kilburn and Warshaw, 1993; Kilburn and Thornton, 1996). Observations in these studies are more uncertain given questions of the representativeness of the referent population, lack of exposure assessment to individual study subjects, and inability to control for possible confounders including alcohol consumption and motivation. Finally, in a presentation of 2 case reports, decrements in motor skills as measured by the grooved pegboard and finger tapping tests were observed (Troster and Ruff, 1990).

Laboratory animal studies of acute or subchronic exposure to TCE observed psychomotor effects, such as loss of righting reflex (Umezu et al., 1997; Shih et al., 2001) and decrements in activity, sensory-motor function, and neuromuscular function (Kishi et al., 1993; Moser et al., 1995; Moser et al., 2003). However, two studies also noted an absence of significant changes in some measures of psychomotor function (Kulig et al., 1987; Albee et al., 2006). In addition, less consistent results have been reported with respect to locomotor activity in rodents. Some studies have reported increased locomotor activity after an acute i.p. dosage (Wolff and Siegmund, 1978) or decreased activity after acute or short term oral gavage dosing (Moser et al., 1995, 2003). No change in activity was observed following exposure through drinking water (Waseem et al., 2001), inhalation (Kulig et al., 1987) or orally during the neurodevelopment period (Fredriksson et al., 1993).

Several neurochemical and molecular changes have been reported in laboratory investigations of TCE toxicity. Kjellstrand et al. (1987) reported inhibition of sciatic nerve regeneration in mice and rats exposed continuously to 150-ppm TCE via inhalation for 24 days. Two studies have reported changes in GABAergic and glutamatergic neurons in terms of GABA or glutamate uptake (Briving et al., 1986) or response to GABAergic antagonistic drugs (Shih et al., 2001) as a result of TCE exposure, with the Briving et al. (1986) conducted at

50 ppm for 12 months. Although the functional consequences of these changes is unclear, Tham et al. (1979, 1984) described central vestibular system impairments as a result of TCE exposure that may be related to altered GABAergic function. In addition, several *in vitro* studies have demonstrated that TCE exposure alters the function of inhibitory ion channels such as receptors for $GABA_A$ glycine, and serotonin (Krasowski and Harrison, 2000; Beckstead et al., 2000; Lopreato et al., 2003) or of voltage-sensitive calcium channels (Shafer et al., 2005).

4.11.1.2. *Kidney Toxicity*

There are few human data pertaining to TCE-related noncancer kidney toxicity. Observation of elevated excretion of urinary proteins in the available studies (Rasmussen et al., 1993a; Brüning et al., 1999a, b; Bolt et al., 2004; Green et al., 2004) indicates the occurrence of a toxic insult among TCE-exposed subjects compared to unexposed controls. Two studies are of subjects with previously diagnosed kidney cancer (Brüning et al., 1999a; Bolt et al., 2004), while subjects in the other studies are disease free. Urinary proteins are considered nonspecific markers of nephrotoxicity and include α1-microglobulin, albumin, and NAG (Price et al., 1996; Lybarger et al., 1999; Price et al., 1999). Four studies measure α1-microglobulin with elevated excretion observed in the German studies (Brüning et al., 1999a, b; Bolt et al., 2004) but not Green et al. (2004). However, Rasmussen et al. (1993a) reported a positive relationship between increasing urinary NAG, another nonspecific marker of tubular toxicity, and increasing exposure duration; and Green et al. (2004) found statistically significant group mean differences in NAG. Observations in Green et al. (2004) provide evidence of tubular damage among workers exposed to trichloroethylene at current occupational levels. Elevated excretion of NAG has also been observed with acute TCE poisoning (Carrieri et al., 2007). Some support for TCE nephrotoxicity in humans is provided by a study of end-stage renal disease in a cohort of workers at Hill Air Force Base (Radican et al., 2006), although subjects in this study were exposed to hydrocarbons, JP-4 gasoline, and solvents in addition to TCE, including 1,1,1-trichloroethane.

Laboratory animal and *in vitro* data provide additional support for TCE nephrotoxicity. Multiple studies with both gavage and inhalation exposure show that TCE causes renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male and female rats and mice (summarized in Section 4.4.4). Further studies with TCE metabolites have demonstrated a potential role for DCVC, TCOH, and TCA in TCE-induced nephrotoxicity. Of these, available data suggest that DCVC induced renal effects most like those of TCE and is formed in sufficient amounts following TCE exposure to account for these effects. TCE or DCVC have also been shown to be cytotoxic to primary cultures of rat and human renal tubular cells (Cummings et al., 2000a, b; Cummings and Lash, 2000).

Overall, multiple lines of evidence support the conclusion that TCE causes nephrotoxicity in the form of tubular toxicity, mediated predominantly through the TCE GSH conjugation product DCVC.

4.11.1.3. *Liver Toxicity*

Few studies on liver toxicity and TCE exposure are found in humans. Of these, three studies reported significant changes in serum liver function tests, widely used in clinical settings in part to identify patients with liver disease, in metal degreasers whose TCE exposure was assessed using urinary trichloro-compounds as a biomarker (Nagaya et al., 1993; Rasmussen et al., 1993; Xu et al., 2009). Two additional studies reported plasma or serum bile acid changes (Neghab et al., 1997; Driscoll et al., 1992). One study of subjects from the TCE subregistry of ATSDR's National Exposure Registry is suggestive of liver disorders but limitations preclude inferences whether TCE caused these conditions is not possible given the study's limitations (Davis et al., 2005). Furthermore, a number of case reports exist of liver toxicity including hepatitis accompanying immune-related generalized skin diseases described as a variation of erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis patients, and hypersensitivity syndrome (Kamijima et al., 2007) in addition to jaundice, hepatomegaly, hepatosplenomegaly, and liver failure TCE-exposed workers (Thiele, 1982; Huang et al., 2002). Cohort studies have examined cirrhosis mortality and either TCE exposure (Blair et al., 1989; Morgan et al., 1998; Boice et al., 1999, 2006; Garabrant et al., 1988; Blair et al., 1998; Ritz et al., 1999; ATSDR, 2004; Radican et al., 2008) or solvent exposure (Leigh and Jiang, 1993), but are greatly limited by their use of death certificates where there is a high degree (up to 50%) of underreporting (Blake et al., 1988), so these null findings do not rule out an effect of TCE on cirrhosis. Overall, while there some evidence exists of liver toxicity as assessed from liver function tests, the data are inadequate for making conclusions regarding causality.

In laboratory animals, TCE exposure is associated with a wide array of hepatotoxic endpoints. Like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids (Bai et al., 1992b; Neghab et al., 1997), although the toxicologic importance of this effect is unclear. Most other effects in laboratory animals have not been studied in humans, but nonetheless provide evidence that TCE exposure leads to hepatotoxicity. These effects include increased liver weight, small transient increases in DNA synthesis, cytomegaly in the form of "swollen" or enlarged hepatocytes, increased nuclear size probably reflecting polyploidization, and proliferation of peroxisomes. Liver weight increases proportional to TCE dose are consistently reported across numerous studies and appear to be accompanied by periportal hepatocellular hypertrophy (Nunes et al., 2001; Tao et al., 2000, Tucker et al., 1982; Goldsworthy and Popp, 1987; Elcombe et al., 1985; Dees and Travis, 1993;

Nakajima et al., 2000; Berman et al., 1995; Melnick et al., 1987; Laughter et al., 2004; Merrick et al., 1989; Goel et al., 1992; Kjellstand et al., 1981, 1983a, b; Buben and O'Flaherty, 1985). There is also evidence of increased DNA synthesis in a small portion of hepatocytes at around 10 days *in vivo* exposure (Mirsalis et al., 1989; Elcombe et al., 1985; Dees and Travis, 1993; Channel et al., 1998). The lack of correlation of hepatocellular mitotic figures with whole liver DNA synthesis or DNA synthesis observed in individual hepatocytes (Elcombe et al., 1985; Dees and Travis, 1993) supports the conclusions that cellular proliferation is not the predominant cause of increased DNA synthesis and that nonparenchymal cells may also contribute to such synthesis. Indeed, nonparenchymal cell activation or proliferation has been noted in several studies (Kjellstrand et al., 1983b; Goel et al., 1992). Moreover, the histological descriptions of TCE-exposed livers are consistent with and, in some cases, specifically note increased polyploidy (Buben and O'Flaherty, 1985). Interestingly, changes in TCE-induced hepatocellular ploidy, as indicated by histological changes in nuclei, have been noted to remain after the cessation of exposure (Kjellstrand et al., 1983a). In regard to apoptosis, TCE has been reported either to have no effect or to cause a slight increase at high doses (Dees and Travis, 1993; Channel et al., 1998). Some studies have also noted effects from dosing vehicle alone (such as corn oil, in particular) not only on liver pathology, but also on DNA synthesis (Merrick et al., 1989; Channel et al., 1998). Available data also suggest that TCE does not induce substantial cytotoxicity, necrosis, or regenerative hyperplasia, as only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers having been reported (Elcombe et al., 1985; Dees and Travis, 1993; Channel et al., 1998). Data on peroxisome proliferation, along with increases in a number of associated biochemical markers, show effects in both mice and rats (Elcombe et al., 1985; Channel et al., 1998; Goldsworthy and Popp, 1987). These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg/d dose appears to be highly variability across strains, with mice on average appearing to be more sensitive.

While it is likely that oxidative metabolism is necessary for TCE-induced effects in the liver, the specific metabolite or metabolites responsible is less clear. TCE, TCA, and DCA exposures have all been associated with induction of changes in liver weight, DNA synthesis, and peroxisomal enzymes. The available data strongly support TCA ***not*** being the sole or predominant active moiety for TCE-induced liver effects, particularly with respect to hepatomegaly. In particular, TCE and TCA dose-response relationships are quantitatively inconsistent, for TCE leads to greater increases in liver/body weight ratios that expected from predicted rates of TCA production (see analysis in Section 4.5.6.2.1). In fact, above a certain dose of TCE, liver/body weight ratios are greater than that observed under any conditions studied so far for TCA. Histological changes and effects on DNA synthesis are generally consistent with

contributions from either TCA or DCA, with a degree of polyploidization, rather than cell proliferation, likely to be significant for TCE, TCA, and DCA.

Overall, TCE, likely through its oxidative metabolites, clearly leads to liver toxicity in laboratory animals, with mice appearing to be more sensitive than other laboratory animal species, but there is only limited epidemiologic evidence of hepatotoxicity being associated with TCE exposure.

4.11.1.4. *Immunotoxicity*

Studies in humans provide evidence of associations between TCE exposure and a number of immunotoxicological endpoints. The relation between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies. A meta-analysis of scleroderma studies (Diot et al., 2002; Garabrant et al., 2003; Nietert et al., 1998) conducted by the U.S. EPA resulted in a statistically significant combined odds ratio for any exposure in men (OR: 2.5, 95% CI: 1.1, 5.4), with a lower relative risk seen in women (OR: 1.2, 95% CI: 0.58, 2.6). The incidence of systemic sclerosis among men is very low (approximately 1 per 100,000 per year), and is approximately 10 times lower than the rate seen in women (Cooper and Stroehla, 2003). Thus, the human data at this time do not allow determination of whether the difference in effect estimates between men and women reflects the relatively low background risk of scleroderma in men, gender-related differences in exposure prevalence or in the reliability of exposure assessment (Messing et al., 2003), a gender-related difference in susceptibility to the effects of TCE, or chance. Changes in levels of inflammatory cytokines were reported in an occupational study of degreasers exposed to TCE (Iavicoli et al., 2005) and a study of infants exposed to TCE via indoor air (Lehmann et al., 2001, 2002).

Experimental studies provide additional support for these effects. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice (Cai et al., 2008; Blossom et al., 2007, 2004; Griffin et al., 2000a, b). With shorter exposure periods, effects include changes in cytokine levels similar to those reported in human studies. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and interestingly, these effects differ somewhat from the "normal" expression in these mice. Immunotoxic effects, including increases in anti-ds DNA antibodies in adult animals, decreased thymus weights, and decreased plaque forming cell response with prenatal and neonatal exposure, have been also reported in B6C3F1 mice, which do not have a known particular susceptibility to autoimmune disease (Gilkeson et al., 2004; Keil et al., 2009; Peden-Adams et al., 2006). Recent mechanistic studies have focused on the roles of various measures of oxidative stress in the induction of these effects by TCE (Wang et al., 2008, 2007b).

There have been a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to TCE, with prevalences as high as 13% of workers in the same location (Kamijima et al., 2008, 2007). Evidence of a treatment-related increase in delayed hypersensitivity response accompanied by hepatic damage has been observed in guinea pigs following intradermal injection (Tang et al., 2008, 2002), and hypersensitivity response was also seen in mice exposed via drinking water pre- and postnatally (gestation Day 0 through to 8 weeks of age) (Peden-Adams et al., 2006).

Human data pertaining to TCE-related immunosuppression resulting in an increased risk of infectious diseases is limited to the report of an association between reported history of bacteria of viral infections in Woburn, Massachusetts (Lagakos, 1986). Evidence of localized immunosuppression, as measured by pulmonary response to bacterial challenge (i.e., risk of Streptococcal pneumonia-related mortality and clearance of Klebsiella bacteria) was seen in an acute exposure study in CD-1 mice (Aranyi et al., 1986). A 4-week inhalation exposure in Sprague-Dawley rats reported a decrease in plaque forming cell response at exposures of 1,000 ppm (Woolhiser et al., 2006).

Overall, the human and animal studies of TCE and immune-related effects provide strong evidence for a role of TCE in autoimmune disease and in a specific type of generalized hypersensitivity syndrome, while there are less data pertaining to immunosuppressive effects.

4.11.1.5. *Respiratory Tract Toxicity*

There are very limited human data on pulmonary toxicity and TCE exposure. Two recent reports of a study of gun manufacturing workers reported asthma-related symptoms and lung function decrements associated with solvent exposure (Cakmak et al., 2004; Saygun et al., 2007), but these studies are limited by multiple solvent exposures and the significant effect of smoking on pulmonary function. Laboratory studies in mice and rats have shown toxicity in the bronchial epithelium, primarily in Clara cells, following acute exposures to TCE by inhalation (see Section 4.7.2.1.1). A few studies of longer duration have reported more generalized toxicity, such as pulmonary fibrosis 90 days after a single 2,000 mg/kg i.p. dose in mice and pulmonary vasculitis after 13-week oral gavage exposures to 2,000 mg/kg/d in rats (Forkert and Forkert, 1994; NTP, 1990). However, respiratory tract effects were not reported in other longer-term studies. Acute pulmonary toxicity appears to be dependent on oxidative metabolism, although the particular active moiety is not known. While earlier studies implicated chloral produced *in situ* by CYP enzymes in respiratory tract tissue was responsible for toxicity (reviewed in Green, 2000), the evidence is inconsistent, and several other possibilities are viable. First, substantial "accumulation" of chloral is unlikely, as it is likely either to be rapidly converted to TCOH in

respiratory tract tissue or to diffuse rapidly into blood and be converted to TCOH in erythrocytes or the liver. Conversely, a role for systemically produced oxidative metabolites cannot be discounted, as CH and TCOH in blood have both been reported following inhalation dosing in mice. In addition, a recent study reported dichloroacetyl chloride protein adducts in the lungs of mice to which TCE was administered by i.p. injection, suggesting dichloroacetyl chloride, which is not believed to be derived from chloral, may also contribute to TCE respiratory toxicity. Although humans appear to have lower overall capacity for enzymatic oxidation in the lung relative to mice, CYP enzymes do reside in human respiratory tract tissue, suggesting that, qualitatively, the respiratory tract toxicity observed in rodents is biologically plausible in humans. However, quantitative estimates of differential sensitivity across species due to respiratory metabolism are highly uncertain due to limited data. Therefore, overall, data are suggestive of TCE causing respiratory tract toxicity, based primarily on short-term studies in mice and rats, and no data suggest that such hazards would be biologically precluded in humans.

4.11.1.6. *Reproductive Toxicity*

Reproductive toxicity related to TCE exposure has been evaluated in human and experimental animal studies for effects in males and females. Only a limited number of studies have examined whether TCE causes female reproductive toxicity. Epidemiologic studies have identified possible associations of TCE exposure with effects on female fertility (Sallmén et al., 1995; ATSDR, 2001) and with menstrual cycle disturbances (ATSDR, 2001; Bardodej and Vyskocil, 1956; Sagawa et al., 1973; Zielinski, 1973). Reduced *in vitro* oocyte fertilizability has been reported as a result of TCE exposure in rats (Berger and Horner, 2003; Wu and Berger, 2007), but a number of other laboratory animal studies did not report adverse effects on female reproductive function (Cosby and Dukelow, 1992; George et al., 1985, 1986; Manson et al., 1984). Overall, there are inadequate data to conclude whether adverse effects on human female reproduction are caused by TCE.

By contrast, a number of human and laboratory animal studies suggest that TCE exposure has the potential for male reproductive toxicity. In particular, human studies have reported TCE exposure to be associated, in several cases statistically-significantly, with increased sperm density and decreased sperm quality (Chia et al., 1996; Rasmussen et al., 1988), altered sexual drive or function (El Gawabi et al., 1973; Saihan et al., 1978; Bardodej and Vyskocil, 1956), or altered serum endocrine levels (Chia et al., 1997; Goh et al., 1998). In addition, three studies that reported measures of fertility did not or could not report changes associated with TCE exposure (ATSDR, 2001; Forkert et al., 2003; Sallmén et al., 1998), although the statistical power of these studies is quite limited. Further evidence of similar effects is provided by several laboratory animal studies that reported effects on sperm (Kumar et al., 2000a, b, 2001;

George et al., 1985; Land et al., 1981; Veeramachaneni et al., 2001), libido/copulatory behavior (George et al., 1986; Zenick et al., 1984; Veeramachaneni et al., 2001), and serum hormone levels (Kumar et al., 2000b; Veeramachaneni et al., 2001). As with the human database, some studies that assessed sperm measures did not report treatment-related alterations (Cosby and Dukelow, 1992; Xu et al., 2004; Zenick et al., 1984; George et al., 1986). Additional adverse effects on male reproduction have also been reported, including histopathological lesions in the testes or epididymides (George et al., 1986; Kumar et al., 2000a, 2001; Forkert et al., 2002; Kan et al., 2007) and altered *in vitro* sperm-oocyte binding or *in vivo* fertilization due to TCE or metabolites (Xu et al., 2004; DuTeaux et al., 2004b). While reduced fertility in rodents was only observed in one study (George et al., 1986), this is not surprising given the redundancy and efficiency of rodent reproductive capabilities. Furthermore, while George et al. (1986) proposed that the adverse male reproductive outcomes observed in rats were due to systemic toxicity, the database as a whole suggests that TCE does induce reproductive toxicity independent of systemic effects. Therefore, overall, the human and laboratory animal data together support the conclusion that TCE exposure poses a potential hazard to the male reproductive system.

4.11.1.7. *Developmental Toxicity*

The relationship between TCE exposure (direct or parental) and adverse developmental outcomes has been investigated in a number of epidemiologic and laboratory animal studies. Prenatal effects examined include death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, small for gestational age, intrauterine growth restriction, decreased postnatal growth), and congenital malformations, in particular eye and cardiac defects. Postnatal developmental outcomes examined include growth and survival, developmental neurotoxicity, developmental immunotoxicity, and childhood cancers.

A few epidemiological studies have reported associations between parental exposure to TCE and spontaneous abortion or perinatal death (Taskinen et al., 1994; Windham et al., 1991; ATSDR, 2001), although other studies reported mixed or null findings (ATSDR, 2006, 2008; Bove, 1996; Bove et al., 1995; Goldberg et al., 1990; Lagakos et al., 1986; Lindbohm et al., 1990; Taskinen et al., 1989). Studies examining associations between TCE exposure and decreased birth weight or small for gestational age have reported small, often nonstatistically significant, increases in risk for these effects (ATSDR, 1998, 2006, 2008; Windham et al., 1991). However, other studies observed mixed or no association (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986; Rodenbeck et al., 2000). While comprising both occupational and environmental exposures, these studies are overall not highly informative due to their small numbers of cases and limited exposure characterization or to the fact that exposures to mixed

solvents were involved. However, a number of laboratory animal studies show analogous effects of TCE exposure in rodents. In particular, pre- or postimplantation losses, increased resorptions, perinatal death, and decreased birth weight have been reported in multiple well-conducted studies in rats and mice (Healy et al., 1982; Kumar et al., 2000a; George et al., 1985, 1986; Narotsky et al., 1995; Narotsky and Kavlock, 1995). Interestingly, the rat studies reporting these effects used Fischer 344 or Wistar rats, while several other studies, all of which used Sprague-Dawley rats, reported no increased risk in these developmental measures (Carney et al., 2006; Hardin et al., 1981; Schwetz et al., 1975). Overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

Epidemiologic data provide some support for the possible relationship between maternal TCE exposure and birth defects in offspring, in particular cardiac defects. Other developmental outcomes observed in epidemiology and experimental animal studies include an increase in total birth defects (AZ DHS, 1988; ATSDR, 2001), CNS defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), oral cleft defects (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986; Lorente et al., 2000), eye/ear defects (Lagakos et al., 1986; Narotsky et al., 1995; Narotsky and Kavlock, 1995), kidney/urinary tract disorders (Lagakos et al., 1986), musculoskeletal birth anomalies (Lagakos et al., 1986), lung/respiratory tract disorders (Lagakos et al., 1986; Das and Scott, 1994), and skeletal defects (Healy et al., 1982). Occupational cohort studies, while not consistently reporting positive results, are generally limited by the small number of observed or expected cases of birth defects (Lorente et al., 2000; Tola et al., 1980; Taskinen et al., 1989).

While only one of the epidemiological studies specifically reported observations of eye anomalies (Lagakos et al., 1986), studies in rats have identified increases in the incidence of fetal eye defects following oral exposures during the period of organogenesis with TCE (Narotsky et al., 1995; Narotsky and Kavlock, 1995) or its oxidative metabolites DCA and TCA (Smith et al., 1989, 1992; Warren et al., 2006). No other developmental or reproductive toxicity studies identified abnormalities of eye development following TCE exposures, which may have been related to the administered dose or other aspects of study design (e.g., level of detail applied to fetal ocular evaluation). Overall, the study evidence suggests a potential for the disruption of ocular development by exposure to TCE and its oxidative metabolites.

The epidemiological studies, while individually limited, as a whole show relatively consistent elevations, some of which were statistically significant, in the incidence of cardiac effects in TCE-exposed populations compared to reference groups (ATSDR, 2001, 2006, 2008; Bove et al., 1995; Bove, 1996; Goldberg et al., 1990; Yauck et al., 2004). Interestingly, Goldberg et al. (1990) noted that the odds ratio for congenital heart disease in offspring declined

from 3-fold to no difference as compared to controls after TCE-contaminated drinking water wells were closed, suggestive of a causal relationship. However, this study reported no significant differences in cardiac lesions between exposed and nonexposed groups (Goldberg et al., 1990). One additional community study reported that, among the 5 cases of cardiovascular anomalies, there was no significant association with TCE (Lagakos et al., 1986), but due to the small number of cases this does not support an absence of effect. In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development, and the initial findings have been confirmed multiple times (Bross et al., 1983; Loeber et al., 1988; Boyer et al., 2000; Drake et al., 2006a, b; Mishima et al., 2006; Rufer et al., 2008). Additionally, administration of TCE and TCE metabolites TCA and DCA in maternal drinking water during gestation has been reported to induce cardiac malformations in rat fetuses (Dawson et al., 1990, 1993; Johnson et al., 1998a, b, 2003, 2005; Smith et al., 1989, 1992; Epstein et al., 1992). However, it is notable that a number of other studies, several of which were well conducted, did not report induction of cardiac defects in rats or rabbits from TCE administered by inhalation (Dorfmueller et al., 1979; Schwetz et al., 1975; Hardin et al., 1981; Healy et al., 1982; Carney et al., 2006) or in rats and mice by gavage (Cosby and Dukelow, 1992; Narotsky et al., 1995; Narotsky and Kavlock, 1995; Fisher et al., 2001).

The potential importance of these effects warrants a more detailed discussion of possible explanations for the apparent inconsistencies in the laboratory animal studies. Many of the studies that did not identify cardiac anomalies used a traditional free-hand section technique on fixed fetal specimens (Dorfmueller et al., 1979; Schwetz et al., 1975; Hardin et al., 1981; Healy et al., 1982). Detection of such anomalies can be enhanced through the use of a fresh dissection technique as described by Staples (1974) and Stuckhardt and Poppe (1984), and this was the technique used in the study by Dawson et al. (1990), with further refinement of the technique used in the positive studies by Dawson et al. (1993) and Johnson et al. (2003, 2005). However, two studies that used the same or similar fresh dissection technique did not report cardiac anomalies (Fisher et al., 2001; Carney et al., 2006), although it has been suggested that differences in experimental design (e.g., inhalation versus gavage versus drinking water route of administration, exposure during organogenesis versus the entire gestational period, or varied dissection or evaluation procedures) may have been contributing factors to the differences in observed response. A number of other limitations in the studies by Dawson et al. (1993) and Johnson et al. (2003, 2005) have been suggested (Hardin et al., 2005; Watson et al., 2006). One concern is the lack of clear dose-response relationship for the incidence of any specific cardiac anomaly or combination of anomalies, a disparity for which no reasonable explanation has been put forth. In addition, analyses on a fetal- rather than litter-basis and the pooling of data collected over an extended period, including nonconcurrent controls, have been criticized. With

respect to the first issue, the study authors provided individual litter incidence data to U.S. EPA for analysis (see Chapter 5, dose-response), and, in response to the second issue, the study authors provided further explanation as to their experimental procedures (Johnson et al., 2004). In sum, while the studies by Dawson et al. (1993) and Johnson et al. (2003, 2005) have significant limitations, there is insufficient reason to dismiss their findings.

Finally, mechanistic studies, particularly based on the avian studies mentioned above, provide additional support for TCE-induced fetal cardiac malformation, particularly with respect to defects involving septal and valvular morphogenesis. As summarized by NRC (2006), there is substantial concordance in the stages and events of cardiac valve formation between mammals and birds. While quantitative extrapolation of findings from avian studies to humans is not possible without appropriate kinetic data for these experimental systems, the treatment-related alterations in endothelial cushion development observed in avian *in ovo* and *in vitro* studies (Boyer et al., 2000; Mishima et al., 2006; Ou et al., 2003) provide a plausible mechanistic basis for defects in septal and valvular morphogenesis observed in rodents, and consequently support the plausibility of cardiac defects induced by TCE in humans.

Postnatal developmental outcomes examined after TCE prenatal and/or postnatal exposure in both humans and experimental animals include developmental neurotoxicity, developmental immunotoxicity, and childhood cancer. Effects on the developing nervous system included a broad array of structural and behavioral alterations in humans (White et al., 1997; Windham et al., 2006; Burg et al., 1995; Burg and Gist, 1997; Bernad et al., 1987; Laslo-Baker et al., 2004; Till et al., 2001; Beppu, 1968; ATSDR, 2003a) and animals (Fredriksson et al., 1993; George et al., 1986; Isaacson and Taylor, 1989; Narotsky and Kavlock, 1995; Noland-Gerbec et al., 1986; Taylor et al., 1985; Westergren et al., 1984; Blossom et al., 2008). Adverse immunological findings in humans following developmental exposures to TCE were reported by Lehmann et al. (2002) and Byers et al. (1988). In mice, alterations in T-cell subpopulations, spleen and/or thymic cellularity, cytokine production, autoantibody levels (in an autoimmune-prone mouse strain), and/or hypersensitivity response were observed after exposures during development (Blossom and Doss, 2007; Blossom et al., 2008; Peden-Adams et al., 2006, 2008), Childhood cancers included leukemia and non-Hodgkin's lymphoma (Morgan and Cassady, 2002; McKinney et al., 1991; Lowengart et al., 1987; Cohn et al., 1994; Cutler et al., 1986; Lagakos et al., 1986; Costas et al., 2002; MA DPH, 1997; Shu et al., 1999; AZ DHS, 1988, 1990a, b, c, 1997), CNS tumors (Morgan and Cassady, 2002; AZ DHS, 1998, 1990a, c, 1997; DeRoos et al., 2001; Peters and Preston-Martin, 1984; Peters et al., 1981, 1985), and total cancers (Morgan and Cassady, 2002; ATSDR, 2006, 2008; AZ DHS, 1988, 1990a, 1997). These outcomes are discussed in the other relevant sections for neurotoxicity, immunotoxicity, and carcinogenesis.

4.11.2. Characterization of Carcinogenicity

In 1995, IARC concluded that trichloroethylene is "probably carcinogenic to humans" (IARC, 1995). In 2000, National Toxicology Program (NTP) concluded that trichloroethylene is "reasonably anticipated to be a human carcinogen" (NTP, 2000). In 2001, the draft U.S. EPA health risk assessment of TCE concluded that TCE was "highly likely" to be carcinogenic in humans. In 2006, a committee of the National Research Council stated that "findings of experimental, mechanistic, and epidemiologic studies lead to the conclusion that trichloroethylene can be considered a potential human carcinogen" (NRC, 2006).

Following U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, based on the available data as of 2009, TCE is characterized as "*Carcinogenic to Humans"* by all routes of exposure. This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer. The human evidence of carcinogenicity from epidemiologic studies of TCE exposure is compelling for lymphoma but less convincing than for kidney cancer, and more limited for liver and biliary tract cancer. Additionally, there are several lines of supporting evidence for TCE carcinogenicity in humans. First, TCE induces site-specific tumors in rodents given TCE by oral gavage and inhalation. Second, toxicokinetic data indicate that TCE absorption, distribution, metabolism, and excretion are qualitatively similar in humans and rodents. Finally, with the exception of a mutagenic MOA for TCE-induced kidney tumors, MOAs have not been established for TCE-induced tumors in rodents, and no mechanistic data indicate that any hypothesized key events are biologically precluded in humans.

4.11.2.1. *Summary Evaluation of Epidemiologic Evidence of Trichloroethylene (TCE) and Cancer*

The available epidemiologic studies provide convincing evidence of a causal association between TCE exposure and cancer. The strongest epidemiologic evidence consists of reported increased risks of kidney cancer, with more limited evidence for lymphoma and liver cancer, in several well-designed cohort and case-control studies (discussed below). The summary evaluation below of the evidence for causality is based on guidelines adapted from Hill (1965) by U.S. EPA (2005), and focuses on evidence related to kidney cancer, lymphoma, and liver cancer.

4.11.2.1.1. ***(a) Consistency of observed association.*** Elevated risks for kidney cancer have been observed across many independent studies. Eighteen studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on job-exposure matrices or biomarker monitoring) and which were judged to have met, to a sufficient degree, the standards of epidemiologic design and analysis, were identified in a systematic review of the epidemiologic

literature. Of the 14 of these studies reporting risks of kidney cancer, most estimated relative risks between 1.1 and 1.9 for overall exposure to TCE. Five of these 14 studies reported statistically significant increased risks either for overall exposure to TCE (Dosemeci et al., 1999; Bruning et al., 2003; Raaschou-Nielsen et al., 2003) or for one of the highest TCE exposure group (Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Charbotel et al., 2006). Thirteen other cohort, case-control, and geographic based studies were given less weight because of their lesser likelihood of TCE exposure and other study design limitations that would decrease statistical power and study sensitivity.

The consistency of association between TCE exposure and kidney cancer is further supported by the results of the meta-analyses of the 14 cohort and case-control studies of sufficient quality and with high probability TCE exposure potential to individual subjects. These analyses observed a statistically significant increased pooled relative risk estimate (RRp) for kidney cancer of 1.25 (95% CI: 1.11, 1.41) for overall TCE. The pooled relative risk were robust and did not change appreciably with the removal of any individual study or with the use of alternate relative risk estimates from individual studies. In addition, there was no evidence for heterogeneity or publication bias.

The consistency of increased kidney cancer relative risk estimates across a large number of independent studies of different designs and populations from different countries and industries argues against chance, bias or confounding as the basis for observed associations. This consistency, thus, provides substantial support for a causal effect between kidney cancer and TCE exposure.

Some evidence of consistency is found between TCE exposure and lymphoma and liver cancer. In a weight-of-evidence review of the lymphoma studies, 16 studies in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on job-exposure matrices or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis were identified. These studies generally reported excess relative risk estimates for lymphoma between 0.8 and 3.1 for overall TCE exposure. Statistically significant elevated relative risk estimates were observed in two cohort (Hansen et al., 2001; Raaschou-Nielsen et al., 2003) and one case-control (Hardell et al., 1994) studies. The other 13 high-quality studies reported elevated relative risk estimates with overall TCE exposure that were not statistically significant. Fifteen additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease study power and sensitivity. The observed lack of association with lymphoma in these studies likely reflects study design and exposure assessment limitations and is not considered inconsistent with the overall evidence on TCE and lymphoma.

Consistency of the association between TCE exposure and lymphoma is further supported by the results of meta-analyses. These meta-analyses found a statistically significant increased pooled relative risk estimate for lymphoma of 1.23 (95% CI: 1.04, 1.44) for overall TCE exposure. This result and its statistical significance were not overly influenced by most individual studies. 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 et al. (2005) mortality RR estimate was substituted with the incidence estimate, resulting in an RRp estimate of 1.19 (95% CI: 1.00, 1.41]).. Some heterogeneity was observed across the 16 studies, though it was not statistically significant ($p = 0.10$). Analyzing the cohort and case-control studies separately resolved most of the heterogeneity, but the result for the pooled case-control studies was only about a 7% increased relative risk estimate and was not statistically significant. The sources of heterogeneity are uncertain but may be the result of some bias associated with exposure assessment and/or disease classification, or from differences between cohort and case-control studies in average TCE exposure. Notably, no heterogeneity was observed in the meta-analysis of the highest exposure group, providing some evidence of exposure misclassification as a source of heterogeneity in the overall analysis. In addition, 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 standard error 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.

There are fewer studies on liver cancer than for kidney cancer and lymphoma. Of nine studies, all of them cohort studies, in which there is a high likelihood of TCE exposure in individual study subjects (e.g., based on job-exposure matrices or biomarker monitoring) and which met, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review, most reported relative risk estimates for liver and gallbladder cancer between 0.5 and 2.0 for overall exposure to TCE. Relative risk estimates were generally based on small numbers of cases or deaths, with the result of wide confidence intervals on the estimates, except for one study (Raaschou-Nielsen et al., 2003). This study has almost 6 times more cancer cases than the next largest study and observed a statistically significant elevated liver and gallbladder cancer risk with overall TCE exposure (RRp = 1.35 [95% CI: 1.03, 1.77]). Ten additional studies were given less weight because of their lesser likelihood of TCE exposure and other design limitations that would decrease statistical power and study sensitivity.

Consistency of the association between TCE exposure and liver cancer is further supported by the results of meta-analyses. These meta-analyses found a statistically significant

increased pooled relative risk estimate for liver and biliary tract cancer of 1.33 (95% CI: 1.09, 1.64) with overall TCE exposure. Although there was no evidence of heterogeneity or publication bias and the pooled estimate was fairly insensitive to the use of alternative relative risk estimates, the statistical significance of the pooled estimate depends heavily on the one large study by Raaschou-Nielsen et al. (2003). However, there were fewer adequate studies available for meta-analysis of liver cancer (9 versus 16 for lymphoma and 14 for kidney), leading to lower statistical power, even with pooling. Moreover, liver cancer is comparatively rarer, with age-adjusted incidences roughly half or less those for kidney cancer or lymphoma; thus, fewer liver cancer cases are generally observed in individual cohort studies.

4.11.2.1.2. ***(b) Strength of the observed association.*** In general, the observed associations between TCE exposure and cancer are modest, with relative risks or odds ratios for overall TCE exposure generally less than 2.0, and higher relative risks or odds ratios for high exposure categories. Among the highest statistically significant relative risks were those reported for kidney cancer in the studies by Henschler et al. (1995) (7.97 [95% CI: 2.59, 8.59]) and Vamvakas et al. (1998) (10.80 [95% CI: 3.36, 34.75]). As discussed in Section 4.5.3., risk magnitude in both studies is highly uncertain due, in part, to possible selection biases, and neither was included in the meta-analyses. However, the findings of these studies were corroborated, though with lower reported relative risks, by later studies which overcame many of their deficiencies, such as Brüning et al. (2003) (2.47 [95% CI: 1.36, 4.49]) and Charbotel et al. (2006, 2009) (2.16 [95% CI: 1.02, 4.60] for the high cumulative exposure group]. In addition, the very high apparent exposure in the subjects of Henschler et al. (1995) and Vamvakas et al. (1998) may have contributed to their reported relative risks being higher than those in other studies. Exposures in most population case-control studies are of lower overall TCE intensity compared to exposures in Brüning et al. (2003) and Charbotel et al. (2006, 2009), and, as would be expected, observed relative risk estimates are lower (1.24 [95% CI: 1.03, 1.49]), Pesch et al., 2000a; 1.30 [95% CI: 0.9, 1.9], Dosemeci et al., 1999). A few high-quality cohort studies reported statistically significant relative risks of approximately 2.0 with highest exposure, including Zhao et al. (2005) (4.9 [95% CI: 1.23, 19.6] for high TCE score), Raaschou-Nielsen et al. (2003) (1.7 [95% CI: 1.1, 2.4] for $\geq$5 year exposure duration, subcohort with higher exposure]), and Charbotel et al. (2006) (2.16 [95% CI: 1.02, 4.60] for high cumulative exposure and 2.73 [95% CI: 1.06, 7.07] for high cumulative exposure plus peaks).

Among the highest statistically significant relative risks reported for lymphoma were those of Hansen et al. (2001) (3.1 [95% CI: 1.3, 6.1]) and Hardell et al. (1994) (7.2 [95% CI: 1.3, 42]), the latter a case-control study whose magnitude of risk is uncertain because of self-reported occupational TCE exposure. However, these findings are corroborated in Seidler et al. (2007)

(2.1 [95% CI: 1.0, 4.88] for high cumulative exposure), a population case-control study with a higher quality exposure assessment approach. Observed relative risk estimates for liver cancer and overall TCE exposure are generally more modest.

Overall, the strength of association between TCE exposure and cancer is not large with overall TCE exposure. Large relative risk estimates are considered strong evidence of causality; however, a modest risk does not preclude a causal association and may reflect a lower level of exposure, an agent of lower potency, or a common disease with a high background level (U.S. EPA, 2005). Modest relative risk estimates have been observed with several well-established human carcinogens such as benzene and secondhand smoke. Chance cannot explain the observed association between TCE and cancer; statistically significant associations are found in a number of the studies that contribute greater weight to the overall evidence, given their design and statistical analysis approaches. In addition, other known or suspected risk factors can not fully explain the observed elevations in kidney cancer relative risks. All kidney cancer case-control studies included adjustment for possible confounding effects of smoking, and some studies included body mass index and hypertension. The associations between kidney cancer and TCE exposure remained in these studies after adjustment for possible known and suspected confounders. Charbotel et al. (2009) observed a nonstatistically significantly kidney cancer risk with exposure to only TCE with cutting fluids (1.11 [95% CI: 0.11, 10.71]) or to only cutting fluids without TCE (1.24 [95% CI: 0.39, 3.93]); however, the finding of a 4-fold higher risk with both cutting fluid and time-weight-average TCE exposure >50 ppm (3.74 [95% CI: 1.32, 10.57]) supports association with TCE. Although direct examination of smoking and other suspected kidney cancer risk factors is usually not possible in cohort studies, confounding is less likely in Zhao et al. (2005), given their use of an internal referent group and adjustment for socioeconomic status, an indirect surrogate for smoking, and other occupational exposures. In addition, the magnitude of the lung cancer risk in Raaschou-Nielsen et al. (2003) suggests a high smoking rate is unlikely and cannot explain their finding on kidney cancer.

Few risk factors are recognized for lymphoma, with the exception of viruses and suspected factors such as immunosuppression or smoking, which are associated with specific lymphoma subtypes. Associations between lymphoma and TCE exposure are based on groupings of several lymphoma subtypes. Three of the six lymphoma case-control studies adjusted for age, sex and smoking in statistical analyses (Miligi et al., 2006; Seidler et al., 2007; Wang et al., 2009), the other three case-control studies presented only unadjusted estimates of the odds ratio. Like for kidney cancer, direct examination of possible confounding in cohort studies is not possible. The use of internal controls in some of the higher quality cohort studies is intended to reduce possible confounding related to lifestyle differences, including smoking habits, between exposed and referent subjects.

Heavy alcohol use and viral hepatitis are established risk factors for liver cancer, with severe obesity and diabetes characterized as a metabolic syndrome associated with liver cancer. Only cohort studies for liver cancer are available, and they were not able to consider these possible risk factors.

4.11.2.1.3. ***(c) Specificity of the observed association.*** Specificity is generally not as relevant as other aspects for judging causality. As stated in the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005), based on our current understanding that many agents cause cancer at multiple sites, and cancers have multiple causes, the absence of specificity does not detract from evidence for a causal effect. Evidence for specificity could be provided by a biological marker in tumors that was specific to TCE exposure. There is some evidence suggesting particular VHL mutations in kidney tumors may be caused by TCE, but uncertainties in these data preclude a definitive conclusion.

4.11.2.1.4. ***(d) Temporal relationship of the observed association.*** Each cohort study was evaluated for the adequacy of the follow-up period to account for the latency of cancer development. The studies with the greatest weight based on study design characteristics (e.g., those used in the meta-analysis) all had adequate follow-up to assess associations between TCE exposure and cancer. Therefore, the findings of those studies are consistent with a temporal relationship.

4.11.2.1.5. ***(e) Biological gradient (exposure-response relationship).*** Exposure-response relationships are examined in the TCE epidemiologic studies only to a limited extent. Many studies examined only overall "exposed" versus "unexposed" groups and did not provide exposure information by level of exposure. Others do not have adequate exposure assessments to confidently distinguish between levels of exposure. For example, many studies used duration of employment as an exposure surrogate; however, this is a poor exposure metric given subjects may have differing exposure intensity with similar exposure duration (NRC, 2006).

Two studies of kidney cancer reported a statistically significant trend of increasing risk with increasing TCE exposure, Zhao et al. (2005) ($p = 0.023$ for trend with TCE score) and Charbotel et al. (2005, 2007) ($p = 0.04$ for trend with cumulative TCE exposure). Charbotel et al. (2007) was specifically designed to examine TCE exposure and had a high-quality exposure assessment. Zhao et al. (2005) also had a relatively well-designed exposure assessment. A positive trend was also observed in one other study (Raaschou-Nielsen et al., 2003, with employment duration).

Biological gradient is further supported by meta-analyses for kidney cancer using only the highest exposure groups and accounting for possible reporting bias, which yielded a higher pooled relative risk estimate (1.53 [95% CI: 1.23, 1.91]) than for overall TCE exposure (1.25 [95% CI: 1.11, 1.41]). Although this analysis uses a subset of studies in the overall TCE exposure analysis, the finding of higher risk in the highest exposure groups, where such groups were available, is consistent with a trend of increased risk with increased exposure.

The lymphoma case-control study of Seidler et al. (2007) reported a statistically significant trend with TCE exposure ($p = 0.03$ for Diffuse B-cell lymphoma trend with cumulative TCE exposure), and lymphoma risk in Boice et al. (1999) appeared to increase with increasing exposure duration ($p = 0.20$ for routine-intermittent exposed subjects). The borderline trend with TCE intensity in the case-control study of Wang et al. (2009) ($p = 0.06$) is consistent with Seidler et al. (2007). As with kidney cancer, further support was provided by meta-analyses using only the highest exposure groups, which yielded a higher pooled relative risk estimate (1.57 [95% CI: 1.27, 1.94]) than for overall TCE exposure (1.23 [95% CI: 1.04, 1.44]). For liver cancer, the meta-analyses using only the highest exposure groups yielded a lower, and nonstatistically significant, pooled estimate for primary liver cancer (1.25 [95% CI: 0.87, 1.79]) than overall TCE exposure (1.28 [95% CI: 0.93, 1.77]). There were no case-control studies on liver cancer and TCE, and the cohort studies generally had few liver cancer cases, making it more difficult to assess exposure-response relationships. The one large study (Raaschou-Nielsen et al., 2003) used only duration of employment, which is an inferior exposure metric.

4.11.2.1.6. ***(f) Biological plausibility.*** TCE metabolism is similar in humans, rats, and mice and results in reactive metabolites. TCE is metabolized in multiple organs and metabolites are systemically distributed. Several oxidative metabolites produced primarily in the liver, including CH, TCA and DCA, are rodent hepatocarcinogens. Two other metabolites, DCVC and DCVG, which can be produced and cleared by the kidney, have shown genotoxic activity, suggesting the potential for carcinogenicity. Kidney cancer, lymphomas, and liver cancer have all been observed in rodent bioassays (see below). The laboratory animal data for liver and kidney cancer are the most robust, corroborated in multiple studies, sexes, and strains, although each has only been reported in a single species and the incidences of kidney cancer are quite low. Lymphomas were only reported to be statistically significantly elevated in a single study in mice, but one additional mouse study reported elevated lymphoma incidence and one rat study reported elevated leukemia incidence. In addition, there is some evidence both in humans and laboratory animals for kidney, liver and immune system noncancer toxicity from TCE exposure. Several hypothesized modes of action have been presented for the rodent tumor findings, although there

are insufficient data to support any one mode of action, and the available evidence does not preclude the relevance of the hypothesized modes of action to humans.

4.11.2.1.7. ***(g) Coherence.*** Coherence is defined as consistency with the known biology. As discussed under biological plausibility, the observance of kidney and liver cancer, and lymphomas in humans is consistent with the biological processing and toxicity of TCE.

4.11.2.1.8. ***(h) Experimental evidence (from human populations).*** Few experimental data from human populations are available on the relationship between TCE exposure and cancer. The only study of a "natural experiment" (i.e., observations of a temporal change in cancer incidence in relation to a specific event) notes that childhood leukemia cases appeared to be more evenly distributed throughout Woburn, MA, after closure of the two wells contaminated with trichloroethylene and other organic solvents (MA DPH, 1997).

4.11.2.1.9. ***(i) Analogy.*** Exposure to structurally related chlorinated solvents such as tetrachloroethylene and dichloromethane have also been associated with kidney, lymphoid, and liver tumors in human, although the evidence for TCE is considered stronger.

4.11.2.1.10.*Conclusion.* In conclusion, based on the weight-of-evidence analysis for kidney cancer and in accordance with U.S. EPA guidelines, TCE is characterized as "Carcinogenic to Humans." This hazard descriptor is used when there is convincing epidemiologic evidence of a causal association between human exposure and cancer. Convincing evidence is found in the consistency of the kidney cancer findings. The consistency of increased kidney cancer relative risk estimates across a large number of independent studies of different designs and populations from different countries and industries provides compelling evidence given the difficulty, a priori, in detecting effects in epidemiologic studies when the relative risks are modest, the cancers are relatively rare, and therefore, individual studies have limited statistical power. This strong consistency argues against chance, bias, and confounding as explanations for the elevated kidney cancer risks. In addition, statistically significant exposure-response trends are observed in high-quality studies. These studies were designed to examine kidney cancer in populations with high TCE exposure intensity. These studies addressed important potential confounders and biases, further supporting the observed associations with kidney cancer as causal. In a meta-analysis of 14 high-quality studies, a statistically significant pooled relative risk estimate was observed for overall TCE exposure (RRp: 1.25 [95% CI: 1.11, 1.41]). The pooled relative risk estimate was greater for the highest TCE exposure groups (RRp: 1.53 [95% CI: 1.23, 1.91]; n = 12 studies). Meta-analyses investigating the influence of individual studies and the sensitivity of

the results to alternate relative risk estimate selections found the pooled relative risk estimates to be highly robust. Furthermore, there was no indication of publication bias or significant heterogeneity. It would require a substantial amount of high-quality negative data to contradict this observed association.

The evidence is less convincing for lymphoma and liver cancer. While the evidence is strong for lymphoma, issues of (non-statistically significant) study heterogeneity, potential publication bias, and weaker exposure-response results contribute greater uncertainty. The evidence is more limited for liver cancer mainly because only cohort studies are available and most of these studies have small numbers of cases.

4.11.2.2. *Summary of Evidence for Trichloroethylene (TCE) Carcinogenicity in Rodents*

Additional evidence of TCE carcinogenicity consists of increased incidences of tumors reported in multiple chronic bioassays in rats and mice. In total, this database identifies some of the same target tissues of TCE carcinogenicity also seen in epidemiological studies, including the kidney, liver, and lymphoid tissues.

Of particular note is the site-concordant finding of TCE-induced kidney cancer in rats. In particular, low, but biologically and sometimes statistically significant, increases in the incidence of kidney tumors were observed in multiple strains of rats treated with TCE by either inhalation or corn oil gavage (Maltoni et al., 1986; NTP, 1988, 1990). For instance, Maltoni et al. (1986) reported that although only 4/130 renal adenocarcinomas in rats in the highest dose group, these tumors had never been observed in over 50,000 Sprague-Dawley rats (untreated, vehicle-treated, or treated with different chemicals) examined in previous experiments in the same laboratory In addition, the gavage study by NCI (1976) and two inhalation studies by Henschler et al. (1980), and Fukuda et al. (1983) each observed one renal adenoma or adenocarcinoma in some dose groups and none in controls. The largest (but still small) incidences were observed in treated male rats, only in the highest dose groups. However, given the small numbers, an effect in females cannot be ruled out. Several studies in rats were limited by excessive toxicity, accidental deaths, or deficiencies in reporting (NCI, 1976; NTP, 1988, 1990). Individually, therefore, these studies provide only suggestive evidence of renal carcinogenicity. Overall, given the rarity of these types of tumors in the rat strains tested and the repeated similar results across experiments and strains, these studies taken together support the conclusion that TCE is a kidney carcinogen in rats, with males being more sensitive than females. No other tested laboratory species (i.e., mice and hamsters) have exhibited increased kidney tumors, although high incidences of kidney toxicity have been reported in mice (NCI, 1976; Maltoni et al., 1986; NTP, 1990). The GSH-conjugation-derived metabolites suspected of mediating TCE-induced kidney carcinogenesis have not been tested in a standard 2-year bioassay, so their role cannot be

confirmed definitively. However, it is clear that GSH conjugation of TCE occurs in humans and that the human kidney contains the appropriate enzymes for bioactivation of GSH conjugates. Therefore, the production of the active metabolites thought to be responsible for kidney tumor induction in rats likely occurs in humans.

Statistically significant increases in TCE-induced liver tumors have been reported in multiple inhalation and gavage studies with male Swiss mice and B6C3F1 mice of both sexes (NCI, 1976; Maltoni et al., 1986; NTP, 1990; Anna et al., 1994; Herren-Freund et al., 1987; Bull et al., 2002). In female Swiss mice, on the other hand, Fukuda et al. (1983), in CD-1 (ICR, Swiss-derived) mice, and Maltoni et al. (1986) both reported small, nonsignificant increases at the highest dose by inhalation. Henschler et al. (1980, 1984) reported no increases in either sex of Han:NMRI (also Swiss-derived) mice exposed by inhalation and ICR/HA (Swiss) mice exposed by gavage. However, the inhalation study (Henschler et al., 1980) had only 30 mice per dose group and the gavage study (Henschler et al., 1984) had dosing interrupted due to toxicity. Studies in rats (NCI, 1976; Henschler et al., 1980; Maltoni et al., 1986; NTP, 1988, 1990) and hamsters (Henschler et al., 1980) did not report statistically significant increases in liver tumor induction with TCE treatment. However, several studies in rats were limited by excessive toxicity or accidental deaths (NCI, 1976; NTP, 1988, 1990), and the study in hamsters only had 30 animals per dose group. These data are inadequate for concluding that TCE lacks hepatocarcinogenicity in rats and hamsters, but are indicative of a lower potency in these species. Moreover, it is notable that a few studies in rats reported low incidences (too few for statistical significance) of very rare biliary- or endothelial-derived tumors in the livers of some treated animals (Fukuda et al., 1983; Henschler et al., 1980; Maltoni et al.,1986). Further evidence for the hepatocarcinogenicity of TCE is derived from chronic bioassays of the TCE oxidative metabolites CH, TCA, and DCA in mice (e.g., George et al., 2000; Leakey et al., 2003a; Bull et al., 1990; DeAngelo et al., 1996, 1999, 2008), all of which reported hepatocarcinogenicity. Very limited testing of these TCE metabolites has been done in rats, with a single experiment reported in both Richmond et al. (1995) and DeAngelo et al. (1996) finding statistically significant DCA-induced hepatocarcinogenicity. With respect to TCA, DeAngelo et al. (1997), often cited as demonstrating lack of hepatocarcinogenicity in rats, actually reported elevated adenoma multiplicity and carcinoma incidence from TCA treatment. However, statistically, the role of chance could not be confidently excluded because of the low number of animals per dose group (20–24 per treatment group at final sacrifice). Overall, TCE and its oxidative metabolites are clearly carcinogenic in mice, with males more sensitive than females and the B6C3F1 strain appearing to be more sensitive than the Swiss strain. Such strain and sex differences are not unexpected, as they appear to parallel, qualitatively, differences in background tumor incidence. Data in other laboratory animal species are limited. Thus, except

for DCA, which is carcinogenic in rats, inadequate evidence exists to evaluate the hepatocarcinogenicity of these compounds in rats or hamsters. However, to the extent that there is hepatocarcinogenic potential in rats, TCE is clearly less potent in the strains tested in this species than in B6C3F1 and Swiss mice.

Additionally, there is more limited evidence for TCE-induced lymphatic cancers in rats and mice, lung tumors in mice, and testicular tumors in rats. With respect to the lymphomas, Henschler et al. (1980) reported statistically significant increases in lymphomas in female Han:NMRI mice treated via inhalation. While Henschler et al. (1980) suggested these lymphomas were of viral origin specific to this strain, subsequent studies reported increased lymphomas in female B6C3F1 mice treated via corn oil gavage (NTP, 1990) and leukemias in male Sprague-Dawley and female August rats (Maltoni et al., 1986; NTP, 1988). However, these tumors had relatively modest increases in incidence with treatment, and were not reported to be increased in other studies. With respect to lung tumors, rodent bioassays have demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE (Fukuda et al., 1983; Maltoni et al., 1988, 1986). Pulmonary tumors were not reported in other species tested (i.e., rats and hamsters; Maltoni et al., 1986, 1988; Fukuda et al., 1983; Henschler et al., 1980). Chronic oral exposure to TCE led to a nonstatistically significant increase in pulmonary tumors in mice but, again, not in rats or hamsters (Henschler et al., 1984; Van Duuren et al., 1979; NCI, 1976; NTP, 1988, 1990; Maltoni et al., 1986). A lower response via oral exposure would be consistent with a role of respiratory metabolism in pulmonary carcinogenicity. Finally, increased testicular (interstitial cell and Leydig cell) tumors have been observed in rats exposed by inhalation and gavage (NTP, 1988, 1990; Maltoni et al., 1986). Statistically significant increases were reported in Sprague-Dawley rats exposed via inhalation (Maltoni et al., 1986) and Marshall rats exposed via gavage (NTP, 1988). In three rat strains, ACI, August, and F344/N, a high (>75%) control rate of testicular tumors was observed, limiting the ability to detect a treatment effect (NTP, 1988, 1990).

In summary, there is clear evidence for TCE carcinogenicity in rats and mice, with multiple studies showing TCE to cause tumors at multiple sites. The apparent lack of site concordance across laboratory animal species may be due to limitations in design or conduct in a number of rat bioassays and/or genuine interspecies differences in sensitivity. Nonetheless, these studies have shown carcinogenic effects across different strains, sexes, and routes of exposure, and site-concordance is not necessarily expected for carcinogens.

4.11.2.3. *Summary of Additional Evidence on Biological Plausibility*

Additional evidence from toxicokinetic, toxicity, and mechanistic studies supports the biological plausibility of TCE carcinogenicity in humans.

4.11.2.3.1. ***Toxicokinetics.*** As described in Chapter 3, there is no evidence of major qualitative differences across species in TCE absorption, distribution, metabolism, and excretion. In particular, available evidence is consistent with TCE being readily absorbed via oral, dermal, and inhalation exposures, and rapidly distributed to tissues via systemic circulation. Extensive *in vivo* and *in vitro* data show that mice, rats, and humans all metabolize TCE via two primary pathways: oxidation by CYPs and conjugation with glutathione via GSTs. Several metabolites and excretion products from both pathways, including TCA, DCA, TCOH, TCOG, NAcDCVC, and DCVG, have been detected in blood and urine from exposed humans was well as from at least one rodent species. In addition, the subsequent distribution, metabolism, and excretion of TCE metabolites are qualitatively similar among species. Therefore, humans possess the metabolic pathways that produce the TCE metabolites thought to be involved in the induction of rat kidney and mouse liver tumors, and internal target tissues of both humans and rodents experience a similar mix of TCE and metabolites.

As addressed in further detail elsewhere (see Chapters 3 and 5), examples of quantitative interspecies differences in toxicokinetics include differences in partition coefficients, metabolic capacity and affinity in various tissues, and plasma binding of the metabolite TCA. These and other differences are addressed through PBPK modeling, which also incorporates physiological differences among species (see Section 3.5), and are accounted for in the PBPK model-based dose-response analyses (see Chapter 5). Importantly, these quantitative differences affect only interspecies extrapolations of carcinogenic potency, and do not affect inferences as to the carcinogenic hazard for TCE. In addition, available data on toxicokinetic differences do not appear sufficient to explain interspecies differences in target sites of TCE carcinogenicity (discussed further in Chapter 5: Dose-Response).

4.11.2.3.2. ***Toxicity and mode of action.*** Many different MOAs have been proposed for TCE-induced carcinogenesis. With respect to genotoxicity, although it appears unlikely that TCE, as a pure compound, causes point mutations, there is evidence for TCE genotoxicity with respect to other genetic endpoints, such as micronucleus formation (see Section 4.2.1.4.4). In addition, as discussed further below, several TCE metabolites have tested positive in genotoxicity assays. The MOA conclusions for specific target organs in laboratory animals are summarized below. Only in the case of the kidney is it concluded that the data are sufficient to support a particular MOA being operative. However, the available evidence do not indicate that qualitative differences between humans and test animals would preclude any of the hypothesized key events in rodents from occurring in humans.

For the kidney, the predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular DCVC, see Section 4.2.5), together with toxicokinetic data consistent with their systemic delivery to and *in situ* formation in the kidney, supports the conclusion that a mutagenic MOA is operative in TCE-induced kidney tumors (see Section 4.4.7.1). Relevant data include demonstration of genotoxicity in available *in vitro* assays of GSH conjugation metabolites and reported kidney-specific genotoxicity after *in vivo* administration of TCE or DCVC. Mutagenicity is a well-established cause of carcinogenicity. While supporting the biological plausibility of this hypothesized MOA, available data on the *VHL* gene in humans or transgenic animals do not conclusively elucidate the role of *VHL* mutation in TCE-induced renal carcinogenesis. Cytotoxicity and compensatory cell proliferation, also presumed to be mediated through metabolites formed after GSH-conjugation of TCE, have also been suggested to play a role in the MOA for renal carcinogenesis, as high incidences of nephrotoxicity have been observed in animals at doses that also induce kidney tumors. Human studies have reported markers for nephrotoxicity at current occupational exposures, although data are lacking at lower exposures. Toxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumors are only observed at low incidences in rats at the highest tested doses. Therefore, nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors. In addition, nephrotoxicity has not been shown to be necessary for kidney tumor induction by TCE in rodents. In particular, there is a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumors induced by TCE. Furthermore, it is not clear if nephrotoxicity is one of several key events in a MOA, if it is a marker for an "upstream" key event (such as oxidative stress) that may contribute independently to both nephrotoxicity and renal carcinogenesis, or if it is incidental to kidney tumor induction. Moreover, while toxicokinetic differences in the GSH conjugation pathway, along with their uncertainty, are addressed through PBPK modeling, no data suggest that any of the proposed key events for TCE-induced kidney tumors rats are precluded in humans. Therefore, TCE-induced rat kidney tumors provide additional support for the convincing human evidence of TCE-induced kidney cancer, with mechanistic data supportive of a mutagenic MOA.

The strongest data supporting the hypothesis of a mutagenic MOA in either the lung or the liver are those demonstrating the genotoxicity of CH (see Section 4.2.4), which is produced in these target organs as a result of oxidative metabolism of TCE. It has been suggested that CH mutagenicity is unlikely to be the cause of TCE hepatocarcinogenicity because the concentrations required to elicit these responses are several orders of magnitude higher that

achieved *in vivo* (Moore and Harrington-Brock, 2000). However, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays *in vitro* and concentrations *in vivo*, as reported *in vivo* CH concentrations are in whole liver homogenate while *in vitro* concentrations are in culture media. The use of i.p. administration, which leads to an inflammatory response, in many other *in vivo* genotoxicity assays in the liver and lung complicates the comparison with carcinogenicity data. Also, it is difficult with the available data to assess the contributions from genotoxic effects of CH along with those from the genotoxic and nongenotoxic effects of other oxidative metabolites (e.g., DCA and TCA). Therefore, while data are insufficient to conclude that a mutagenic MOA mediated by CH is operant, a mutagenic MOA in the liver or lung, either mediated by CH or by some other oxidative metabolite of TCE, cannot be ruled out.

A second MOA hypothesis for TCE-induced liver tumors involves activation of the PPARα receptor. Clearly, *in vivo* administration of TCE leads to activation of PPARα in rodents and likely does so in humans as well (based on *in vitro* data for TCE and its oxidative metabolites). However, the evidence as a whole does not support the view that PPAR-α is the sole operant MOA mediating TCE hepatocarcinogenesis. Although metabolites of TCE activate PPARα, the data on the subsequent elements in the hypothesized MOA (e.g., gene regulation, cell proliferation, apoptosis, and selective clonal expansion), while limited, indicate significant differences between PPAR-α agonists such as Wy-14643 and TCE or its metabolites. For example, compared with other agonists, TCE induces transient as opposed to persistent increases in DNA synthesis; increases (or is without effect on), as opposed to decreases, apoptosis; and induces a different H-ras mutation frequency or spectrum. These data support the view that mechanisms other than PPARα activation may contribute to these effects; besides PPARα activation, the other hypothesized key events are nonspecific, and available data (e.g., using knockout mice) do not indicate that they are solely or predominantly dependent on PPARα. A second consideration is whether certain TCE metabolites (e.g., TCA) that activate PPAR-α are the sole contributors to its carcinogenicity. As summarized above (see Section 4.11.1.3), TCA is not the only metabolite contributing to the observed noncancer effects of TCE in the liver. Other data also suggest that multiple metabolites may also contribute to the hepatic carcinogenicity of TCE. Liver phenotype experiments, particularly those utilizing immunostaining for c-Jun, support a role for both DCA and TCA in TCE-induced tumors, with strong evidence that TCA cannot solely account for the characteristics of TCE-induced tumors (e.g., Bull et al., 2002). In addition, H-ras mutation frequency and spectrum of TCE-induced tumors more closely resembles that of spontaneous tumors or of those induced by DCA, and were less similar in comparison to that of TCA-induced tumors. The heterogeneity of TCE-induced tumors is similar to that observed to be induced by a diversity carcinogens including those that do not activate

PPAR-α, and to that observed in human liver cancer. Taken together, the available data indicate that, rather than being solely dependent on a single metabolite (TCA) and/or molecular target (PPAR-α) multiple TCE metabolites and multiple toxicity pathways contribute to TCE-induced liver tumors.

Other considerations as well as new data published since the NRC (2006) review are also pertinent to the liver tumor MOA conclusions. It is generally acknowledged that, qualitatively, there are no data to support the conclusion that effects mediated by the PPAR-α receptor that contribute to hepatocarcinogenesis would be biologically precluded in humans (Klaunig et al., 2003; NRC, 2006). It has, on the other hand, been argued that due to quantitative toxicokinetic and toxicodynamic differences, the hepatocarcinogenic effects of chemicals activating this receptor are "unlikely" to occur in humans (Klaunig et al., 2003; NRC, 2006); however, several lines of evidence strongly undermine the confidence in this assertion. With respect to toxicokinetics, as discussed above, quantitative differences in oxidative metabolism are accounted for in PBPK modeling of available *in vivo* data, and do not support interspecies differences of a magnitude that would preclude hepatocarcinogenic effects based on toxicokinetics alone. With respect to the MOA proposed by Klaunig et al. (2003), recent experiments have demonstrated that PPAR-α activation and the sequence of key events in the hypothesized MOA are not sufficient to induce hepatocarcinogenesis (Yang et al., 2007). Moreover, the demonstration that the PPAR-α agonist DEHP induces tumors in PPAR-α-null mice supports the view that the events comprising the hypothesized MOA are not necessary for liver tumor induction in mice by this PPARα agonist (Ito et al., 2007). Therefore, several lines of evidence, including experiments published since the NRC (2006) review, call into question the scientific validity of using the PPAR-α MOA hypothesis as the basis for evaluating the relevance to human carcinogenesis of rodent liver tumors (Guyton et al., 2009).

In summary, available data support the conclusion that the MOA for TCE-induced liver tumors in laboratory animals is not known. However, a number of qualitative similarities exist between observations in TCE-exposed mice and what is known about the etiology and induction of human hepatocellular carcinomas. Polyploidization, changes in glycogen storage, inhibition of GST-zeta, and aberrant DNA methylation status, which have been observed in studies of mice exposed to TCE or its oxidative metabolites, are all either clearly related to human carcinogenesis or are areas of active research as to their potential roles (PPARα activation is discussed below). The mechanisms by which TCE exposure may interact with known risk factors for human hepatocellular carcinomas are not known. However, available data do not suggest that TCE exposure to mice results in liver tumors that are substantially different in terms of their phenotypic characteristics either from human hepatocellular carcinomas or from rodent liver tumors induced by other chemicals.

Comparing various other, albeit relatively nonspecific, tumor characteristics between rodent species and humans provides additional support to the biologic plausibility of TCE carcinogenicity. For example, in the kidney and the liver, the higher incidences of background and TCE-induced tumors in male rats and mice, respectively, as compared to females parallels the observed higher human incidences in males for these cancers (Ries et al., 2008). For the liver, while there is a lower background incidence of liver tumors in humans than in rodents, in the United States there is an increasing occurrence of liver cancer associated with several factors, including viral hepatitis, higher survival rates for cirrhosis, and possibly diabetes (reviewed in El-Serag, 2007). In addition, Leakey et al. (2003) reported that increased body weight in B6C3F1 mice is strongly associated with increased background liver tumor incidences, although the mechanistic basis for this risk factor in mice has not been established. Nonetheless, it is interesting that recent epidemiologic studies have suggested obesity, in addition to associated disorders such as diabetes and metabolic syndrome, as a risk factor for human liver cancer (El-Serag, 2007; El-Serag and Rudolph, 2007). Furthermore, the phenotypic and morphologic heterogeneity of tumors seen in the human liver is qualitatively similar to descriptions of mouse liver tumors induced by TCE exposure, as well as those observed from exposure to a variety of other chemical carcinogens. These parallels suggest similar pathways (e.g., for cell signaling) of carcinogenesis may be active in mice and humans and support the qualitative relevance of mouse models of liver to human liver cancer.

For mouse lung tumors, MOA hypotheses have centered on TCE metabolites produced via oxidative metabolism *in situ*. As discussed above, the hypothesis that the mutagenicity of reactive intermediates or metabolites (e.g., CH) generated during CYP metabolism contributes to lung tumors cannot be ruled out, although available data are inadequate to conclusively support this MOA. An alternative MOA has been posited involving other effects of such oxidative metabolites, particularly CH, including cytotoxicity and regenerative cell proliferation. Experimental support for this alternative hypothesis remains limited, with no data on proposed key events in experiments of duration 2 weeks or longer. While the data are inadequate to support this MOA hypothesis, the data also do not suggest that any proposed key events would be biologically plausible in humans. Furthermore, the focus of the existing MOA hypothesis involving cytotoxicity has been CH, and, as summarized above (see Section 4.11.1.5), other metabolites may contribute to respiratory tract noncancer toxicity or carcinogenicity. In sum, the MOA for mouse lung tumors induced by TCE is not known.

A MOA subsequent to *in situ* oxidative metabolism, whether involving mutagenicity, cytotoxicity, or other key events, may also be relevant to other tissues where TCE would undergo CYP metabolism. For instance, CYP2E1, oxidative metabolites, and protein adducts have been reported in the testes of rats exposed to TCE, and, in some rat bioassays, TCE

exposure increased the incidence of rat testicular tumors. However, inadequate data exist to adequately define a MOA hypothesis for this tumor site.

4.11.3. Characterization of Factors Impacting Susceptibility

As discussed in more detail in Section 4.10, there is some evidence that certain subpopulations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, pre-existing health status, and lifestyle factors and nutrition status.

Examination of early lifestages includes exposures such as transplacental transfer (Beppu, 1968; Laham, 1970; Withey and Karpinski, 1985; Ghantous et al., 1986; Helliwell and Hutton, 1950) and breast milk ingestion (Fisher et al., 1990, 1997; Pellizzari et al., 1982; Hamada and Tanaka, 1995), early lifestage-specific toxicokinetics, PBPK models (Fisher et al., 1989, 1990), and differential outcomes in early lifestages such as developmental cardiac defects. Although there is more information on susceptibility to TCE during early lifestages than on susceptibility during later lifestages or for other populations with potentially increased susceptibility, there remain a number of uncertainties regarding children's susceptibility. Improved PBPK modeling for using childhood parameters for early lifestages as recommended by the NRC (2006), and validation of these models will aid in determining how variations in metabolic enzymes affect TCE metabolism. In particular, the NRC states that it is prudent to assume children need greater protection than adults, unless sufficient data are available to justify otherwise (NRC, 2006). Because the weight of evidence supports a mutagenic MOA for TCE carcinogenicity in the kidney (see Section 4.4.7), and there is an absence of chemical-specific data to evaluate differences in carcinogenic susceptibility, early-life susceptibility should be assumed and the ADAFs should be applied, in accordance with the Supplemental Guidance (discussed further in Chapter 5).

Fewer data are available on later lifestages, although there is suggestive evidence to indicate that older adults may experience increased adverse effects than younger adults (Mahle et al., 2007; Rodriguez et al., 2007). In general, more studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential life stage-related TCE toxicity.

Examination of gender-specific susceptibility includes toxicokinetics, PBPK models (Fisher et al., 1998), and differential outcomes. Gender differences observed are likely due to variation in physiology and exposure.

Genetic variation likely has an effect on the toxicokinetics of TCE. In particular, differences in CYP2E1 activity may affect susceptibility of TCE due to effects on production of toxic metabolites (Kim and Ghanayem, 2006; Lipscomb et al., 1997; Povey et al., 2001; Yoon et

al., 2007). GST polymorphisms could also play a role in variability in toxic response (Brüning et al., 1997; Wiesenhütter et al., 2007), as well as other genotypes, but these have not been sufficiently tested. Differences in genetic polymorphisms related to the metabolism of TCE have also been observed among various race/ethnic groups (Inoue et al., 1989; Sato et al., 1991b).

Pre-existing diminished health status may alter the response to TCE exposure. Individuals with increased body mass may have an altered toxicokinetic response (Clewell et al., 2000; Sato, 1993; Sato et al., 1991b; Monster et al., 1979; McCarver et al., 1998; Davidson and Beliles, 1991; Lash et al., 2000) resulting in changes the internal concentrations of TCE or in the production of toxic metabolites. Other conditions, including diabetes and hypertension, are risk factors for some of the same health effects that have been associated with TCE exposure, such as renal cell carcinoma. However, the interaction between TCE and known risk factors for human diseases is not known, and further evaluation of the effects due to these factors is needed.

Lifestyle and nutrition factors examined include alcohol consumption, tobacco smoking, nutritional status, physical activity, and socioeconomic status. In particular, alcohol intake has been associated with metabolic inhibition (altered CYP2E1 expression) of TCE in both humans and experimental animals (Bardodej and Vyskocil, 1956; Barret et al., 1984; McCarver et al., 1998; Müller et al., 1975; Sato, 1993; Sato et al., 1980, 1981, 1983, 1991a; Stewart et al., 1974; Kaneko et al., 1994; Larson and Bull, 1989; Nakajima et al., 1988, 1990, 1992b; Okino et al., 1991; Sato and Nakajima, 1985; White and Carlson, 1981). In addition, such factors have been associated with increased baseline risks for health effects associated with TCE, such as kidney cancer (e.g., smoking) and liver cancer (e.g., alcohol consumption). However, the interaction between TCE and known risk factors for human diseases is not known, and further evaluation of the effects due to these factors is needed.

In sum, there is some evidence that certain subpopulations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, pre-existing health status, and lifestyle factors and nutrition status. However, except in the case of toxicokinetic variability characterized using the PBPK model described in Section 3.5, there are inadequate chemical-specific data to quantify the degree of differential susceptibility due to such factors.