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TOXICOLOGICAL REVIEW

OF

TRICHLOROETHYLENE

CHAPTER 6

(CAS No. 79-01-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

This section summarizes the human hazard potential for TCE. For extensive discussions and references, see Chapter 2 for exposure information, Chapter 3 for toxicokinetics and PBPK modeling, and Sections 4.1–4.9 for the epidemiologic and experimental studies of TCE noncancer and cancer toxicity. Section 4.10 summarizes information on susceptibility, and Section 4.11 provides a more detailed summary and references for noncancer toxicity and carcinogenicity.

6.1.1. Exposure (see Chapter 2)

TCE is a volatile compound with moderate water solubility. Most TCE produced today is used for metal degreasing. The highest environmental releases are to the air. Ambient air monitoring data suggest that mean levels have remained fairly constant since 1999 at about 0.3 μg/m^3 (0.06 ppb). As discussed in Chapter 2, in 2006, ambient air monitors (n = 258) had annual means ranging from 0.03 to 7.73 μg/m^3 with a median of 0.13 μg/m^3 and an overall average of 0.23 μg/m^3. Indoor levels are commonly ≥3 times higher than outdoor levels due to releases from building materials and consumer products. Vapor intrusion is a likely significant source in situations where residences are located near soils or groundwater with high contamination levels and sparse indoor air sampling had detected TCE levels ranging from 1 to 140 μg/m^3. TCE is among the most common groundwater contaminants and the one present in the highest concentration in a summary of groundwater analyses reported in 1982. The median level of TCE in groundwater, based on a large survey by the USGS for 1985–2001, is 0.15 μg/L. It has also been detected in a wide variety of foods in the 1–100 μg/kg range. None of the environmental sampling has been done using statistically based national surveys. However, a substantial amount of air and groundwater data have been collected allowing reasonably well-supported estimates of typical daily intakes by the general population: inhalation—13 μg/day and water ingestion—0.2 μg/day. The limited food data suggest an intake of about 5 μg/day, but this must be considered preliminary. Higher exposures have occurred to various occupational groups, particularly with vapor degreasing that has the highest potential for exposure because vapors can escape into the work place. For example, past studies of aircraft workers have shown short-term peak exposures in the hundreds of ppm (>500,000 μg/m^3) and long-term exposures in the low tens of ppm (>50,000 μg/m^3). Occupational exposures have likely decreased in recent years due to better release controls, improvements in worker protection, and substituting other solvents for TCE.

Exposure to a variety of TCE-related compounds, which include metabolites of TCE and other parent compounds that produce similar metabolites, can alter or enhance TCE metabolism and toxicity by generating higher internal metabolite concentrations than would result from TCE exposure by itself. Available estimates suggest that exposures to most of these TCE-related compounds are comparable to or greater than TCE itself.

6.1.2. Toxicokinetics and PBPK Modeling (see Chapter 3 and Appendix A)

TCE is a lipophilic compound that readily crosses biological membranes. Exposures may occur via the oral, dermal, and inhalation routes, with evidence for systemic availability from each route. TCE can also be transferred transplacentally and through breast milk ingestion. TCE is rapidly and nearly completely absorbed from the gut following oral administration, and animal studies indicate that exposure vehicle may impact the time course of absorption: oily vehicles may delay absorption, whereas aqueous vehicles result in a more rapid increase in blood concentrations. See Section 3.1 for additional discussion of TCE absorption.

Following absorption to the systemic circulation, TCE distributes from blood to solid tissues by each organ's solubility. This process is mainly determined by the blood:tissue partition coefficients, which are largely determined by tissue lipid content. Adipose partitioning is high, so adipose tissue may serve as a reservoir for TCE, and accumulation into adipose tissue may prolong internal exposures. TCE attains high concentrations relative to blood in the brain, kidney, and liver—all of which are important target organs of toxicity. TCE is cleared via metabolism mainly in three organs: the kidney, liver, and lungs. See Section 3.2 for additional discussion of TCE distribution.

The metabolism of TCE is an important determinant of its toxicity. Metabolites are generally thought to be responsible for toxicity-especially for the liver and kidney. Initially, TCE may be oxidized via CYP isoforms or conjugated with GSH by GST enzymes. While CYP2E1 is generally accepted to be the CYP isoform most responsible for TCE oxidation, others forms may also contribute. There are conflicting data as to which GST isoforms are responsible for TCE conjugation, with one rat study indicating alpha-class GSTs and another rat study indicating mu and pi-class GST. The balance between oxidative and conjugative metabolites generally favors the oxidative pathway, especially at lower concentrations, and inhibition of CYP-dependent oxidation in vitro increases GSH conjugation in renal preparations. However, different investigators have reported considerably different rates for TCE conjugation in human liver and kidney cell fractions, perhaps due to different analytical methods. The inferred flux through the GSH pathway differs by >4 orders of magnitude across data sets. While the available data are consistent with the higher values being overestimates, the degree of overestimation is unclear, and differing results may be attributable to true interindividual variation. Overall, there remains significant uncertainty in the quantitative estimation of TCE GSH conjugation. See Section 3.3 for additional discussion of TCE metabolism.

Once absorbed, TCE is excreted primarily either in breath as unchanged TCE or carbon dioxide [CO_2], or in urine as metabolites. Minor pathways of elimination include excretion of metabolites in saliva, sweat, and feces. Following oral administration or upon cessation of inhalation exposure, exhalation of unmetabolized TCE is a major elimination pathway. Initially, elimination of TCE upon cessation of inhalation exposure demonstrates a steep concentration-time profile: TCE is rapidly eliminated in the minutes and hours postexposure, and then the rate of elimination via exhalation decreases. Following oral or inhalation exposure, urinary elimination of parent TCE is minimal, with urinary elimination of the metabolites, TCA and TCOH, accounting for the bulk of the absorbed dose of TCE. See Section 3.4 for additional discussion of TCE excretion.

As part of this assessment, a comprehensive Bayesian PBPK model-based analysis of the population toxicokinetics of TCE and its metabolites was developed in mice, rats, and humans (also reported in Chiu et al., 2009). This analysis considered a wider range of physiological, chemical, in vitro, and in vivo data than any previously published analysis of TCE. The toxicokinetics of the "population average," its population variability, and their uncertainties are characterized and estimates of experimental variability and uncertainty are included in this analysis. The experimental database included separate sets for model calibration and evaluation for rats and humans; fewer data were available in mice, and were all used for model calibration. Local sensitivity analyses confirm that the calibration data inform the value of most model parameters, with the remaining parameters either informed by substantial prior information or having little sensitivity with respect to dose metric predictions. The total combination of these approaches and PBPK analysis substantially supports the model predictions. In addition, the approach employed yields an accurate characterization of the uncertainty in metabolic pathways for which available data were sparse or relatively indirect, such as GSH conjugation and respiratory tract metabolism. Key conclusions from the model predictions include: (1) as expected, TCE is substantially metabolized, primarily by oxidation at doses below saturation; (2) GSH conjugation and subsequent bioactivation in humans appear to be 10–100-fold greater than previously estimated; and (3) mice had the greatest rate of respiratory tract oxidative metabolism compared to rats and humans. However, there are uncertainties as to the accuracy of the analytical method used for some of the available in vivo data on GSH conjugation. Because these data are highly influential, the PBPK modeling results for the flux of GSH conjugation should be interpreted with caution. Thus, there is lower confidence in the accuracy of GSH conjugation predictions as compared to other dose-metrics, such as those related to the parent compound, total metabolism, or oxidative metabolites. The predictions of the PBPK model are subsequently used in noncancer and cancer dose-response analyses for inter- and intraspecies extrapolation of toxicokinetics (see Section 6.2, below). See Section 3.5 and Appendix A for additional discussion of and details about PBPK modeling of TCE and metabolites.

6.1.3. Noncancer Toxicity

This section summarizes the weight of evidence for TCE noncancer toxicity. Based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for noncancer toxicity to the CNS, kidney, liver, immune system, male reproductive system, and developing fetus. The evidence is more limited for TCE toxicity to the respiratory tract and female reproductive system. The conclusions pertaining to specific endpoints within these tissues and systems are summarized below.

6.1.3.1. Neurological Effects (see Sections 4.3 and 4.11.1.1 and Appendix D)

Both human and animal studies have associated TCE exposure with effects on several neurological domains. Multiple epidemiologic studies in different populations have reported abnormalities in trigeminal nerve function in association with TCE exposure. Two small studies did not report an association between TCE exposure and trigeminal nerve function. However, statistical power was limited, exposure misclassification was possible, and, in one case, methods for assessing trigeminal nerve function were not available. As a result, these studies do not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment. Laboratory animal studies have also demonstrated TCE-induced changes in the morphology of the trigeminal nerve following short-term exposures in rats. However, one study reported no significant changes in TSEP in rats exposed to TCE for 13 weeks. See Section 4.3.1 for additional discussion of studies of alterations in nerve conduction and trigeminal nerve effects. Human chamber, occupational, and geographic-based/drinking water studies have consistently reported subjective symptoms such as headaches, dizziness, and nausea, which are suggestive of vestibular system impairments. One study reported changes in nystagmus threshold (a measure of vestibular system function) following an acute TCE exposure. There are only a few laboratory animal studies relevant to this neurological domain, with reports of changes in nystagmus, balance, and handling reactivity. See Section 4.3.3 for additional discussion of TCE effects on vestibular function. Fewer and more limited epidemiologic studies are suggestive of TCE exposure being associated with delayed motor function, and changes in auditory, visual, and cognitive function or performance (see

Sections 4.3.2, 4.3.4, 4.3.5, and 4.3.6). Acute and subchronic animal studies show disruption of the auditory system, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Animal studies suggest that while the effects on the auditory system lead to permanent function impairments and histopathology, effects on the visual system may be reversible with termination of exposure. 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 (see Section 4.3.9). An association between TCE exposure and sleep changes has also been demonstrated in rats (see Section 4.3.7). 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 (see Section 4.3.6). Gestational exposure to TCE in humans has been reported to be associated with neurodevelopmental abnormalities including neural tube defects, encephalopathy, impaired cognition, aggressive behavior, and speech and hearing impairment. Developmental neurotoxicological changes have also been observed in animals including aggressive behaviors following an in utero exposure to TCE and a suggestion of impaired cognition as noted by decreased myelination in the CA1 hippocampal region of the brain. See Section 4.3.8 for additional discussion of developmental neurological effects of TCE. Therefore, overall, the strongest neurological evidence of human toxicological hazard is for changes in trigeminal nerve function or morphology and impairment of vestibular function, based on both human and experimental studies, while fewer and more limited evidence exists for delayed motor function, changes in auditory, visual, and cognitive function or performance, and neurodevelopmental outcomes.

6.1.3.2. Kidney Effects (see Sections 4.4.1, 4.4.4, 4.4.6, and 4.11.1.2)

Kidney toxicity has also been associated with TCE exposure in both human and animal studies. There are few human data pertaining to TCE-related noncancer kidney toxicity; however, several available studies reported elevated excretion of urinary proteins, considered nonspecific markers of nephrotoxicity, among TCE-exposed subjects compared to unexposed controls. While some of these studies include subjects previously diagnosed with kidney cancer, other studies report similar results in subjects who are disease free. Some additional support for TCE nephrotoxicity in humans is provided by two studies of ESRD; a study reporting a greater incidence of ESRD in TCE-exposed workers as compared to unexposed controls and a second study reporting a greater risk for progression from IgA or membranous nephropathy glomerulonephritis to ESRD and TCE-exposure. See Section 4.4.1 for additional discussion of human data on the noncancer kidney effects of TCE. Laboratory animal and in vitro data provide additional support for TCE nephrotoxicity. TCE causes renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male and female rats and mice following either oral or inhalation exposure. In rats, the pathology of TCE-induced nephrotoxicity appears distinct from age-related nephropathy. Increased kidney weights have also been reported in some rodent studies. See Section 4.4.4 for additional discussion of laboratory animal data on the noncancer kidney effects of TCE. 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 are most similar to those of TCE and that DCVC 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. See Section 4.4.6 for additional discussion on the role of metabolism in the noncancer kidney effects of TCE. 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.

6.1.3.3. Liver Effects (see Sections 4.5.1, 4.5.3, 4.5.4, 4.5.6, and 4.11.1.3, and Appendix E)

Liver toxicity has also been associated with TCE exposure in both human and animal studies. Although there are few human studies on liver toxicity and TCE exposure, several available studies have reported TCE exposure to be associated with significant changes in serum liver function tests, widely used in clinical settings in part to identify patients with liver disease, or changes in plasma or serum bile acids. Additional, more limited human evidence for TCE induced liver toxicity includes reports suggesting an association between TCE exposure and liver disorders, and case reports of liver toxicity including hepatitis accompanying immune-related generalized skin diseases, jaundice, hepatomegaly, hepatosplenomegaly, and liver failure in TCE-exposed workers. Cohort studies examining cirrhosis mortality and either TCE exposure or solvent exposure are generally null, but these studies cannot rule out an association with TCE because of their use of death certificates where there is a high degree (up to 50%) of underreporting. Overall, while some evidence exists of liver toxicity as assessed from liver function tests, the data are inadequate for making conclusions regarding causality. See Section 4.5.1 for additional discussion of human data on the noncancer liver effects of TCE. In rats and mice, TCE exposure causes hepatomegaly without concurrent cytotoxicity. Like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids, although the toxicological importance of this effect is unclear. Other effects in the rodent liver include 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. Available data also suggest that TCE does not induce substantial cytotoxicity, necrosis, or regenerative hyperplasia, since only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers have been reported. These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg/day dose appears to be highly variable across strains, with mice on average appearing to be more sensitive. See Sections 4.5.3 and 4.5.4 for additional discussion of laboratory animal data on the noncancer liver effects of TCE. 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. However, the available data are strongly inconsistent with TCA being the sole or predominant active moiety for TCE-induced liver effects, particularly with respect to hepatomegaly. See Section 4.5.6 for additional discussion on the role of metabolism in the noncancer liver effects of TCE. 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.

6.1.3.4. Immunological Effects (see Sections 4.6.1.1, 4.6.2, and 4.11.1.4)

Effects related the immune system have also been associated with TCE exposure in both human and animal studies. A relationship between systemic autoimmune diseases, such as scleroderma, and occupational exposure to TCE has been reported in several recent studies, and a meta-analysis of scleroderma studies resulted in a statistically significant combined OR for any exposure in men (OR [OR]: 2.5, 95% CI: 1.1, 5.4), with a lower RR seen in women (OR: 1.2, 95% CI: 0.58, 2.6). The human data at this time do not allow a 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, a gender-related difference in susceptibility to the effects of TCE, or chance. Additional human evidence for the immunological effects of TCE includes studies reporting TCE-associated changes in levels of inflammatory cytokines in occupationally-exposed workers and infants exposed via indoor air at air concentrations typical of such exposure scenarios (see Section 6.1.1); a large number of case reports (mentioned above) of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis; and a reported association between increased history of infections and exposure to TCE contaminated drinking water. See Section 4.6.1.1 for additional discussion of human data on the immunological effects of TCE. Immunotoxicity has also been reported in experimental rodent studies of TCE. Numerous studies have demonstrated accelerated autoimmune responses in autoimmune-prone mice, including changes in cytokine levels similar to those reported in human studies, with more severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, manifesting at longer exposure periods. Immunotoxic effects have been also reported in $B6C3F_1$ mice, which do not have a known particular susceptibility to autoimmune disease. Developmental immunotoxicity in the form of hypersensitivity responses have been reported in TCE-treated guinea pigs and mice via drinking water pre- and postnatally. Evidence of localized immunosuppression has also been reported in mice and rats. See Section 4.6.2 for additional discussion of laboratory animal data on the immunological effects of TCE. 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.

6.1.3.5. Respiratory Tract Effects (see Sections 4.7.1.1, 4.7.2.1, 4.7.3, and 4.11.1.5)

The very few human data on TCE and pulmonary toxicity are too limited for drawing conclusions (see Section 4.7.1.1), but laboratory studies in mice and rats have shown toxicity in the bronchial epithelium, primarily in Clara cells, following acute exposures to TCE (see Section 4.7.2.1). A few studies of longer duration have reported more generalized toxicity, such as pulmonary fibrosis in mice and pulmonary vasculitis in rats. 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 in toxicity, the evidence is inconsistent and several other possibilities are viable. 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. See Section 4.7.3 for additional discussion of the role of metabolism in the noncancer respiratory tract toxicity of TCE. Therefore, overall, data are suggestive of TCE causing respiratory tract toxicity, based primarily on short-term studies in mice and rats, with available human data too few and limited to add to the weight of evidence for pulmonary toxicity.

6.1.3.6. Reproductive Effects (see Sections 4.8.1 and 4.11.1.6)

A number of human and laboratory animal studies suggest that TCE exposure has the potential for male reproductive toxicity, with a more limited number of studies examining female reproductive toxicity. Human studies have reported TCE exposure to be associated (in all but one case statistically-significantly) with increased sperm density and decreased sperm quality, altered sexual drive or function, or altered serum endocrine levels. Measures of male fertility, however, were either not reported or were reported to be unchanged with TCE exposure, though the statistical power of the available studies is quite limited. Epidemiologic studies have identified possible associations of TCE exposure with effects on female fertility and with menstrual cycle disturbances, but these data are fewer than those available for male reproductive toxicity. See Section 4.8.1.1 for additional discussion of human data on the reproductive effects of TCE. Evidence of similar effects, particularly for male reproductive toxicity, is provided by several laboratory animal studies that reported effects on sperm, libido/copulatory behavior, and serum hormone levels, although some studies that assessed sperm measures did not report treatment-related alterations. Additional adverse effects on male reproduction have also been reported, including histopathological lesions in the testes or epididymides and altered in vitro sperm-oocyte binding or in vivo fertilization due to TCE or metabolites. While reduced fertility in rodents was only observed in one study, this is not surprising given the redundancy and efficiency of rodent reproductive capabilities. In addition, although the reduced fertility observed in the rodent study was originally attributed to systemic toxicity, the database as a whole suggests that TCE does induce reproductive toxicity independent of systemic effects. Fewer data are available in rodents on female reproductive toxicity. While in vitro oocyte fertilizability has been reported to be reduced as a result of TCE exposure in rats, a number of other laboratory animal studies did not report adverse effects on female reproductive function. See Section 4.8.1.2 for additional discussion of laboratory animal data on the reproductive effects of TCE. Very limited data are available to elucidate the mode of action for these effects, though some aspects of a putative mode of action (e.g., perturbations in testosterone biosynthesis) appear to have some commonalities between humans and animals (see Section 4.8.1.3.2). Together, the human and laboratory animal data support the conclusion that TCE exposure poses a potential hazard to the male reproductive system, but are more limited with regard to the potential hazard to the female reproductive system.

6.1.3.7. Developmental Effects (see Sections 4.8.3 and 4.11.1.7)

The relationship between TCE exposure (direct or parental) and developmental toxicity has been investigated in a number of epidemiologic and laboratory animal studies. Postnatal developmental outcomes examined include developmental neurotoxicity (addressed above with neurotoxicity), developmental immunotoxicity (addressed above with immunotoxicity), and childhood cancers. Prenatal effects examined include death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, SGA, IUGR, decreased postnatal growth), and congenital malformations, in particular cardiac defects. Some epidemiological studies have reported associations between parental exposure to TCE and spontaneous abortion or perinatal death, and decreased birth weight or SGA, although other studies reported mixed or null findings. While comprising both occupational and environmental exposures, these studies are overall not highly informative due to the small numbers of cases and limited exposure characterization or to the fact that exposures were to a mixture of solvents. See Section 4.8.3.1 for additional discussion of human data on the developmental effects of TCE. However, multiple well-conducted studies in rats and mice show analogous effects of TCE exposure: pre- or postimplantation losses, increased resorptions, perinatal death, and decreased birth weight. Interestingly, the rat studies reporting these effects used F344 or Wistar rats, while several other studies, all of which used Sprague-Dawley rats, reported no increased risk in these developmental measures, suggesting a strain difference in susceptibility. See Section 4.8.3.2 for additional discussion of laboratory animal data on the developmental effects of TCE. Therefore, 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.

With respect to congenital malformations, epidemiology and experimental animal studies of TCE have reported increases in total birth defects, CNS defects, oral cleft defects, eye/ear defects, kidney/urinary tract disorders, musculoskeletal birth anomalies, lung/respiratory tract disorders, skeletal defects, and cardiac defects. Human occupational cohort studies, while not consistently reporting positive results, are generally limited by the small number of observed or expected cases of birth defects. While only one of the epidemiological studies specifically reported observations of eye anomalies, studies in rats have identified increases in the incidence of fetal eye defects following oral exposures during the period of organogenesis with TCE or its oxidative metabolites, DCA and TCA. The epidemiological studies, while individually limited, as a whole show relatively consistent elevations, some of which were statistically significant, in the incidence of cardiac defects in TCE-exposed populations compared to reference groups. 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. Additionally, administration of TCE and its metabolites, TCA and DCA, in maternal drinking water during gestation has been reported to induce cardiac malformations in rat fetuses. It is notable that a number of other studies, several of which were well-conducted, did not report induction of cardiac defects in rats, mice, or rabbits in which TCE was administered by inhalation or gavage. However, many of these studies used a traditional free-hand section technique on fixed fetal specimens, and a fresh dissection technique that can enhance detection of anomalies was used in the positive studies by Dawson et al. (1993) and Johnson et al. (2005, 2003). Nonetheless, two studies that used the same or similar fresh dissection technique did not report cardiac anomalies. Differences in other aspects of experimental design may have been contributing factors to the differences in observed response. In addition, mechanistic studies, such as the treatment-related alterations in endothelial cushion development observed in avian in ovo and in vitro studies, 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. Therefore, while the studies by Dawson et al. (1993) and Johnson et al. (2003) 2005) have significant limitations, including the lack of clear dose-response relationship for the incidence of any specific cardiac anomaly and the pooling of data collected over an extended period, there is insufficient reason to dismiss their findings. See Section 4.8.3.3.2 for additional discussion of the conclusions with respect to TCE-induced cardiac malformations. Therefore, overall, based on weakly suggestive, but overall consistent, epidemiologic data, in combination with evidence from experimental animal and mechanistic studies, it can be concluded that TCE exposure poses a potential hazard for congenital malformations, including cardiac defects, in offspring.

6.1.4. Carcinogenicity (see Sections 4.1, 4.2, 4.4.2, 4.4.5, 4.4.7, 4.5.2, 4.5.5, 4.5.6, 4.5.7, 4.6.1.2, 4.6.2.4, 4.7.1.2, 4.7.2.2, 4.7.4, 4.8.2, 4.9, and 4.11.2, and Appendices B and C)

Following EPA (2005b) *Guidelines for Carcinogen Risk Assessment*, based on the available data as of 2010, 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 consistency of increased kidney cancer RR 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 RRs are modest and the cancers are relatively rare, and therefore, individual studies have limited statistical power. This strong consistency of the epidemiologic data on TCE and kidney cancer argues against chance, bias, and confounding as explanations for the elevated kidney cancer risks. In addition, statistically significant exposure-response trends were observed in high-quality studies. These studies were conducted in populations with high TCE exposure intensity or had the ability to identify TCE-exposed subjects with high confidence. These studies addressed important potential confounders and biases, further supporting the observed associations with kidney cancer as causal. See Section 4.4.2 for additional discussion of the human epidemiologic data on TCE exposure and kidney cancer. In a meta-analysis of 15 studies with high exposure potential, a statistically significant RRm estimate was observed for overall TCE exposure (RRm: 1.27 [95% CI: 1.13, 1.43]). The RRm estimate was greater for the highest TCE exposure groups (RRm: 1.58 [95% CI: 1.28, 1.96]; n = 13 studies). Meta-analyses investigating the influence of individual studies and the sensitivity of the results to alternate RR estimate selections found the RRm estimates to be highly robust. Furthermore, there was no indication of publication bias or significant heterogeneity across the 15 studies. It would require a substantial amount of negative data from informative studies (i.e., studies having a high likelihood of TCE exposure in individual study subjects and which meet, to a sufficient degree, the standards of epidemiologic design and analysis in a systematic review) to contradict this observed association. See Section 4.4.2.5 and Appendix C for additional discussion of the kidney cancer meta-analysis.

The human evidence of carcinogenicity from epidemiologic studies of TCE exposure is strong for NHL but less convincing than for kidney cancer. Studies with high exposure potential generally reported excess RR estimates, with statistically significant increases in three studies with overall TCE exposure, and a statistically significant increase in the high TCE exposure group and statistically significant trend in a fourth study (see Section 4.6.1.2). The consistency of the association between TCE exposure and NHL is further supported by the results of meta-analyses (see Section 4.6.1.2.2 and Appendix C). A statistically significant RRm estimate was observed for overall TCE exposure (RRm: 1.23 [95% CI: 1.07, 1.42]; n = 17 studies), and, as with kidney cancer, the RRm estimate was greater for the highest TCE exposure groups (RRm: 1.43 [95% CI: 1.13, 1.82]; n = 13 studies) than for overall TCE exposure. Sensitivity analyses indicated that these results and their statistical significance were not overly influenced by any single study or choice of individual (study-specific) risk estimates, and in all of the influence and sensitivity analyses, the RRm estimate was statistically significantly increased. Some heterogeneity was observed, particularly between cohort and case-control studies, but it was not statistically significant. In addition, there was some evidence of potential publication bias. Thus, while the evidence is strong for NHL, issues of study heterogeneity, potential publication bias, and weaker exposure-response results contribute greater uncertainty.

The evidence is more limited for liver and biliary tract cancer mainly because only cohort studies are available and most of these studies have small numbers of cases due the comparative rarity of liver and biliary tract cancer. While most studies with high exposure potential reported excess RR estimates, they were generally based on small numbers of cases or deaths, with the result of wide CIs on the estimates. The low number of liver cancer cases in the available studies made assessing exposure-response relationships difficult. See Section 4.5.2 for additional discussion of the human epidemiologic data on TCE exposure and liver cancer. Consistency of the association between TCE exposure and liver cancer is supported by the results of meta-analyses (see Section 4.5.2 and Appendix C). These meta-analyses found a statistically significant increased RRm estimate for liver and biliary tract cancer of 1.29 (95% CI: 1.07, 1.56; n = 9 studies) with overall TCE exposure; but the meta-analyses using only the highest exposure groups yielded a lower, and nonstatistically significant, summary estimate for primary liver cancer (1.28 [95% CI: 0.93, 1.77], n = 8 studies). Although there was no evidence of heterogeneity or publication bias and the summary estimates were fairly insensitive to the use of alternative RR estimates, the statistical significance of the summary estimates depends heavily on the one large study by Raaschou-Nielsen et al. (2003). There were fewer adequate studies with high exposure potential available for meta-analysis of liver cancer (9 vs. 17 for NHL and 15 for kidney), leading to lower statistical power, even with pooling. Thus, while there is epidemiologic evidence of an association between TCE exposure and liver cancer, the much more limited database, both in terms of number of available studies and number of cases within studies, contributes to greater uncertainty as compared to the evidence for kidney cancer or NHL.

In addition to the body of evidence pertaining to kidney cancer, NHL, and liver cancer, the available epidemiologic studies also provide more limited evidence of an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia. Differences between these sets of data and the data for kidney cancer, NHL, and liver cancer are observations from fewer numbers of studies, a mixed pattern of observed risk estimates, and the general absence of exposure-response data from the studies using a quantitative TCE-specific exposure measure.

There are several other lines of supporting evidence for TCE carcinogenicity in humans by all routes of exposure. First, multiple chronic bioassays in rats and mice have reported increased incidences of tumors with TCE treatment via inhalation and gavage, including tumors in the kidney, liver, and lymphoid tissues − target tissues of TCE carcinogenicity also seen in epidemiological studies. Of particular note is the site-concordant finding of low, but biologically and sometimes statistically significant, increases in the incidence of kidney tumors in multiple strains of rats treated with TCE by either inhalation or corn oil gavage (see Section 4.4.5). The increased incidences were only detected at the highest tested doses, and were greater in male than female rats; although, notably, pooled incidences in females from five rat strains tested by NTP (NTP, 1990, 1988) resulted in a statistically significant trend. Although these studies have shown limited increases in kidney tumors, and several individual studies have a number of limitations, given the rarity of these tumors as assessed by historical controls and the repeatability of this result across studies and strains, these are considered biologically significant. Therefore, while individual studies provide only suggestive evidence of renal carcinogenicity, the database as a whole supports 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, with no adequate explanation for these species differences (particularly with mice, which have been extensively tested). With respect to the liver, TCE and its oxidative metabolites CH, TCA, and DCA are clearly carcinogenic in mice, with strain and sex differences in potency that 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 $B6C3F_1$ and Swiss mice. See Section 4.5.5 for additional discussion of laboratory animal data on TCE-induced liver tumors. Additionally, there is more limited evidence for TCE-induced lymphohematopoetic cancers in rats and mice, lung tumors in mice, and testicular tumors in rats. With respect to the lymphohematopoietic cancers, two studies in mice reported increased incidences of lymphomas in females of two different strains, and two studies in rats reported leukemias in males of one strain and females of another. However, these tumors had relatively modest increases in incidence with treatment, and were not reported to be increased in other studies. See Section 4.6.2.4 for additional discussion of laboratory animal data on TCE-induced lymphohematopoetic tumors. With respect to lung tumors, rodent bioassays have demonstrated a statistically significant increase in pulmonary tumors in mice following chronic inhalation exposure to TCE, and nonstatistically significant increases in mice exposed orally; but pulmonary tumors were not reported in other species tested (i.e., rats and hamsters) (see Section 4.7.2.2). Finally, increased testicular (interstitial or Leydig cell) tumors have been observed in multiple studies of rats exposed by inhalation and gavage, although in some cases, high (> 75%) control rates of testicular tumors in rats limited the ability to detect a treatment effect. See Section 4.8.2.2 for additional discussion of laboratory animal data on TCE-induced tumors of the reproductive system. Overall, TCE is clearly carcinogenic in rats and mice. The apparent lack of site concordance across laboratory animal studies may be due to limitations in design or conduct in a number of rat bioassays and/or genuine interspecies differences in qualitative or quantitative sensitivity (i.e., potency). Nonetheless, these studies have shown carcinogenic effects across different strains, sexes, and routes of exposure, and site-concordance is not necessarily expected for carcinogens. Of greater import is the finding that there is site-concordance between the main cancers observed in TCE-exposed humans and those observed in rodent studies—in particular, cancers of the kidney, liver, and lymphoid tissues.

A second line of supporting evidence for TCE carcinogenicity in humans consists of toxicokinetic data indicating that TCE is well absorbed by all routes of exposure, and that TCE absorption, distribution, metabolism, and excretion are qualitatively similar in humans and rodents. As summarized above, there is evidence that TCE is systemically available, distributes to organs and tissues, and undergoes systemic metabolism from all routes of exposure. Therefore, although the strongest evidence from epidemiologic studies largely involves inhalation exposures, the evidence supports TCE carcinogenicity being applicable to all routes of exposure. In addition, there is no evidence of major qualitative differences across species in TCE absorption, distribution, metabolism, and excretion. 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 GSH via GSTs. Several metabolites and excretion products from both pathways have been detected in blood and urine from exposed humans as 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. See Sections 3.1–3.4 for additional discussion of TCE toxicokinetics. Quantitative interspecies differences in toxicokinetics do exist, and are addressed through PBPK modeling (see Section 3.5 and Appendix A). Importantly, these quantitative differences affect only interspecies extrapolations of carcinogenic potency, and do not affect inferences as to the carcinogenic hazard for TCE.

Finally, available mechanistic data do not suggest a lack of human carcinogenic hazard from TCE exposure. In particular, these data do not suggest qualitative differences between humans and test animals that would preclude any of the hypothesized key events in the carcinogenic mode of action 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), together with toxicokinetic data consistent with their systemic delivery to and in situ formation in the kidney, supports the conclusion that a mutagenic mode of action is operative in TCE-induced kidney tumors. While supporting the biological plausibility of this hypothesized mode of action, 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, similarly presumed to be mediated through metabolites formed after GSH-conjugation of TCE, have also been suggested to play a role in the mode of action for renal carcinogenesis, as high incidences of nephrotoxicity have been observed in animals at doses that induce kidney tumors. Human studies have reported markers for nephrotoxicity at current occupational exposures, although data are lacking at lower exposures. Nephrotoxicity 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 mode of action, 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. Therefore, although the data are consistent with the hypothesis that cytotoxicity and regenerative proliferation contribute to TCE-induced kidney tumors, the weight of evidence is not as strong as the support for a mutagenic mode of action. 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 in rats are precluded in humans. See Section 4.4.7 for additional discussion of the mode of action for TCE-induced kidney tumors. 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 mode of action.

With respect to other tumor sites, data are insufficient to conclude that any of the other hypothesized modes of action are operant. In the liver, a mutagenic mode of action mediated by CH, which has evidence for genotoxic effects, or some other oxidative metabolite of TCE cannot be ruled out, but data are insufficient to conclude it is operant. A second mode-of-action 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. However, the evidence as a whole does not support the view that PPARα is the sole operant mode of action mediating TCE hepatocarcinogenesis. Rather, there is evidential support for multiple TCE metabolites and multiple toxicity pathways contributing to TCE-induced liver tumors. Furthermore, recent experiments have demonstrated that PPARα activation and the sequence of key events in the hypothesized mode of action are not sufficient to induce hepatocarcinogenesis (Yang et al., 2007). Moreover, the demonstration that the PPARα agonist di(2-ethylhexyl) phthalate induces tumors in PPARα-null mice supports the view that the events comprising the hypothesized PPARα activation mode of action are not necessary for liver tumor induction in mice by this PPARα agonist (Ito et al., 2007). See Section 4.5.7 for additional discussion of the mode of action for TCE-induced liver tumors. For mouse lung tumors, as with the liver, a mutagenic mode of action involving CH has also been hypothesized, but there are insufficient data to conclude that it is operant. A second mode-of-action hypothesis for mouse lung tumors has been posited involving other effects of oxidative metabolites including cytotoxicity and regenerative cell proliferation, but experimental support remains limited, with no data on proposed key events in experiments of duration two weeks or longer. See Section 4.7.4 for additional discussion of the mode of action for TCE-induced lung tumors. A mode of action 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 mode-of-action hypothesis for this tumor site (see Section 4.8.2.3 for additional discussion of the mode of action for TCE-induced testicular tumors).

6.1.5. Susceptibility (see Sections 4.10 and 4.11.3)

There is some evidence that certain populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, and lifestyle factors and nutrition status. Factors that affect early lifestage susceptibility include exposures such as transplacental transfer and breast milk ingestion, early lifestage-specific toxicokinetics, and differential outcomes in early lifestages such as developmental cardiac defects (see Section 4.10.1). Because the weight of evidence supports a mutagenic mode of action being operative 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 (see summary below in Section 6.2.2.5). 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 due to greater tissue distribution of TCE. In general, more studies specifically designed to evaluate effects in early and later lifestages are needed in order to more fully characterize potential lifestage-related TCE toxicity. Gender-specific (see Section 4.10.2.1) differences also exist in toxicokinetics (e.g., cardiac outputs, percent body fat, expression of metabolizing enzymes) and susceptibility to toxic endpoints (e.g., gender-specific effects on the reproductive system, gender differences in baseline risks to endpoints such as scleroderma or liver cancer). Genetic variation (see Section 4.10.2.2) likely has an effect on the toxicokinetics of TCE. Increased CYP2E1 activity and GST polymorphisms may influence susceptibility of TCE due to effects on production of toxic metabolites or may play a role in variability in toxic response. Differences in genetic polymorphisms related to the metabolism of TCE have also been observed among various race/ethnic groups (see Section 4.10.2.3). Preexisting diminished health status (see Section 4.10.2.4) may alter the response to TCE exposure. Individuals with increased body mass may have an altered toxicokinetic response due to the increased uptake of TCE into fat. Other conditions that may alter the response to TCE exposure include diabetes and hypertension, and lifestyle and nutrition factors (see Section 4.10.2.5) such as alcohol consumption, tobacco smoking, nutritional status, physical activity, and SES status. Alcohol intake has been associated with inhibition of TCE metabolism in both humans and experimental animals. In addition, such conditions have been associated with increased baseline risks for health effects also associated with TCE, such as kidney cancer and liver cancer. 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 populations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting 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.

6.2. DOSE-RESPONSE ASSESSMENT

This section summarizes the major conclusions of the dose-response analysis for TCE noncancer effects and carcinogenicity, with more detailed discussions in Chapter 5.

6.2.1. Noncancer Effects (see Section 5.1)

6.2.1.1. Background and Methods

As summarized above, based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for noncancer toxicity to the CNS, kidney, liver, immune system, male reproductive system, and developing fetus. The evidence is more limited for TCE toxicity to the respiratory tract and female reproductive system.

Dose-response analysis for a noncancer endpoint generally involves two steps: (1) the determination of a POD derived from a BMD,[61] a NOAEL, or a LOAEL, and (2) adjustment of the POD by endpoint/study-specific “uncertainty factors” (UFs), accounting for adjustments and uncertainties in the extrapolation from the study conditions to conditions of human exposure.

Because of the large number of noncancer health effects associated with TCE exposure and the large number of studies reporting on these effects, in contrast to toxicological reviews for chemicals with smaller databases of studies, a formal, quantitative screening process (see Section 5.1) was used to reduce the number of endpoints and studies to those that would best inform the selection of the *critical effects* for the inhalation RfC and oral RfD.[62] As described in Section 5.1, for all studies described in Chapter 4 which reported adverse noncancer health effects and provided quantitative dose-response data, PODs on the basis of applied dose,

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[61]More precisely, it is the benchmark dose lower bound (BMDL), i.e., the (one-sided) 95% lower confidence bound on the dose corresponding to the benchmark response (BMR) for the effect, that is used as the POD.
[62]In EPA noncancer health assessments, the RfC [RfD] is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation [daily oral] exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark concentration [dose], with uncertainty factors generally applied to reflect limitations of the data used.

adjusted by endpoint/study-specific UFs, were used to develop candidate RfCs (cRfCs) and candidate RfDs (cRfDs) intended to be protective for each endpoint individually. Candidate critical effects − those with the lowest cRfCs and cRfDs taking into account the confidence in each estimate − were selected within each of the following health effect domains: (1) neurological, (2) kidney; (3) liver; (4) immunological; (5) reproductive; and (6) developmental. For each of these candidate critical effects, the PBPK model developed in Section 3.5 was used for interspecies, intraspecies, and route-to-route extrapolation on the basis of internal dose to develop PBPK model-based PODs. Plausible internal dose-metrics were selected based on what is understood about the role of different TCE metabolites in toxicity and the mode of action for toxicity. These PODs were then adjusted by endpoint/study-specific UFs, taking into account the use of the PBPK model, to develop PBPK model-based candidate RfCs (p-cRfCs) and candidate RfDs (p-cRfDs). The most sensitive cRfCs, p-cRfCs, cRfDs, and p-cRfDs were then evaluated, taking into account the confidence in each estimate, to arrive at overall candidate RfCs and RfDs for each health effect type. Then, the RfC and RfD for TCE were selected so as to be protective of the most sensitive effects. In contrast to the approach used in most previous assessments, in which the RfC and RfD are each based on a single critical effect, the final RfC and RfD for TCE were based on multiple critical effects that resulted in very similar candidate RfC and RfD values at the low end of the full range of values. This approach was taken here because it provides robust estimates of the RfC and RfD and because it highlights the multiple effects that are all yielding very similar candidate values.

6.2.1.2. Uncertainties and Application of UFs (see Sections 5.1.1 and 5.1.4)

An underlying assumption in deriving a reference value for a noncancer effect is that the dose-response relationship has a threshold. Thus, a fundamental uncertainty is the validity of that assumption. For some effects, in particular effects on very sensitive processes (e.g., developmental processes) or effects for which there is a nontrivial background level and even small exposures may contribute to background disease processes in more susceptible people, a practical threshold (i.e., a threshold within the range of environmental exposure levels of regulatory concern) may not exist.

Nonetheless, under the assumption of a threshold, the desired exposure level to have as a reference value is the maximum level at which there is no appreciable risk for an adverse effect in sensitive subgroups (of humans). However, because it is not possible to know what this level is, UFs are used to attempt to address quantitatively various aspects, depending on the data set, of qualitative uncertainty.

First there is uncertainty about the POD for the application of UFs. Conceptually, the POD should represent the maximum exposure level at which there is no appreciable risk for an adverse effect in the study population under study conditions (i.e., the threshold in the dose-response relationship). Then, the application of the relevant UFs is intended to convey that exposure level to the corresponding exposure level for sensitive human subgroups exposed continuously for a lifetime. In fact, it is again not possible to know that exposure level even for a laboratory study because of experimental limitations (e.g., the power to detect an effect, dose spacing, measurement errors, etc.), and crude approximations like the NOAEL or a BMDL are used. If a LOAEL is used as the POD, then the LOAEL-to-NOAEL UF is applied as an adjustment factor to better approximate the desired exposure level (threshold), although the necessary extent of adjustment is unknown. The standard value for the LOAEL-to-NOAEL UF is 10, although sometimes a value of 3 is used if the effect is considered minimally adverse at the response level observed at the LOAEL or is an early marker for an adverse effect. For one POD in this assessment, a value of 30 was used for the LOAEL-to-NOAEL UF because the incidence rate for the adverse effect was ≥90% at the LOAEL.

If a BMDL is used as the POD, then there are uncertainties regarding the appropriate dose-response model to apply to the data, but these should be minimal if the modeling is in the observable range of the data. There are also uncertainties about what BMR to use to best approximate the desired exposure level (threshold, see above). For continuous endpoints, in particular, it is often difficult to identify the level of change that constitutes the "cut-point" for an adverse effect. Sometimes, to better approximate the desired exposure level, a BMR somewhat below the observable range of the data is selected. In such cases, the model uncertainty is increased, but this is a trade-off to reduce the uncertainty about the POD not being a good approximation for the desired exposure level.

For each of these types of PODs, there are additional uncertainties pertaining to adjustments to the administered exposures (doses). Typically, administered exposures (doses) are converted to equivalent continuous exposures (daily doses) over the study exposure period under the assumption that the effects are related to concentration × time, independent of the daily (or weekly) exposure regimen (i.e., a daily exposure of 6 hours to 4 ppm is considered equivalent to 24 hours of exposure to 1 ppm). However, the validity of this assumption is generally unknown, and, if there are dose-rate effects, the assumption of concentration times time ($C \times t$) equivalence would tend to bias the POD downwards. Where there is evidence that administered exposure better correlates to the effect than equivalent continuous exposure averaged over the study exposure period (e.g., visual effects), administered exposure was not adjusted. For the PBPK analyses in this assessment, the actual administered exposures are taken into account in the PBPK modeling, and equivalent daily values (averaged over the study exposure period) for the dose-metrics are obtained (see above, Section 5.1.3.2). Additional uncertainties about the PBPK-based estimates include uncertainties about the appropriate dose-metric for each effect, although, for some effects, there was better information about relevant dose-metrics than for others, and uncertainties in the PBPK model predictions for the dose-metrics in humans, particularly for GSH conjugation (see Section 5.1.3.1).

There is also uncertainty about the other UFs. The human variability UF is, to some extent, an adjustment factor because, for more sensitive people, the dose-response relationship shifts to lower exposures. But there is uncertainty about the extent of the adjustment required (i.e., about the distribution of human susceptibility). Therefore, in the absence of data on a susceptible population(s) or on the distribution of susceptibility in the general population, an UF of 10 is generally used, which breaks down (approximately) to a factor of 3 for pharmacokinetic variability and a factor of 3 for pharmacodynamic variability. This standard value was used for all of the PODs based on applied dose in this assessment with the exception of the PODs for a few immunological effects that were based on data from a sensitive (autoimmune-prone) mouse strain. For those PODs, an UF of 3 (reflecting pharmacokinetics only) was used for human variability. The PBPK analyses in this assessment attempt to account for the pharmacokinetic portion of human variability using human data on pharmacokinetic variability. For PBPK model-based candidate reference values, the pharmacokinetic component of this UF was omitted. A quantitative uncertainty analysis of the PBPK derived dose-metrics used in the assessment is presented in Section 5.1.4.2. There is still uncertainty regarding the susceptible subgroups for TCE exposure and the extent of pharmacodynamic variability.

If the data used to determine a particular POD are from laboratory animals, an interspecies extrapolation UF is used. This UF is also, to some extent, an adjustment factor for the expected scaling for toxicologically equivalent doses across species (i.e., according to body weight to the ¾ power for oral exposures). However, there is also uncertainty about the true extent of interspecies differences for specific noncancer effects from specific chemical exposures. For oral exposures, the standard value for the interspecies UF is 10, which can be viewed as breaking down (approximately) to a factor of 3 for the "adjustment" (nominally pharmacokinetics) and a factor of 3 for the "uncertainty" (nominally pharmacodynamics). For inhalation exposures for systemic toxicants, such as TCE, for which the blood:air partition coefficient in laboratory animals is greater than that in humans, no adjustment across species is generally assumed for fixed air concentrations (ppm equivalence; U.S. EPA, 1994a), and the standard value for the interspecies UF is 3, reflecting only "uncertainty" (nominally pharmacodynamics). The PBPK analyses in this assessment attempt to account for the "adjustment" portion of interspecies extrapolation using rodent pharmacokinetic data to estimate internal doses for various dose-metrics. Equal doses of these dose-metrics, appropriately scaled, are then assumed to convey equivalent risk across species. For PBPK model-based candidate reference values, the "adjustment" component of this UF was omitted. With respect to the “uncertainty” component, quantitative uncertainty analyses of the PBPK-derived dose-metrics used in the assessment are presented in Section 5.1.4.2. However, these only address the pharmacokinetic uncertainties in a particular dose-metric, and there is still uncertainty regarding the true dose-metrics. Nor do the PBPK analyses address the uncertainty in either cross-species pharmacodynamic differences (i.e., about the assumption that equal doses of the appropriate dose-metric convey equivalent risk across species for a particular endpoint from a specific chemical exposure) or in cross-species pharmacokinetic differences not accounted for by the PBPK model dose-metrics (e.g., departures from the assumed interspecies scaling of clearance of the active moiety, in the cases where only its production is estimated). A value of 3 is typically used for the “uncertainty” about cross-species differences, and this generally represents true uncertainty because it is usually unknown, even after adjustments have been made to account for the expected interspecies differences, whether humans have more or less susceptibility, and to what degree, than the laboratory species in question.

RfCs and RfDs apply to lifetime exposure, but sometimes the best (or only) available data come from less-than-lifetime studies. Lifetime exposure can induce effects that may not be apparent or as large in magnitude in a shorter study; consequently, a dose that elicits a specific level of response from a lifetime exposure may be less than the dose eliciting the same level of response from a shorter exposure period. If the effect becomes more severe with increasing exposure, then chronic exposure would shift the dose-response relationship to lower exposures, although the true extent of the shift is unknown. PODs based on subchronic exposure data are generally divided by a subchronic-to-chronic UF, which has a standard value of 10. If there is evidence suggesting that exposure for longer time periods does not increase the magnitude of an effect, a lower value of 3 or 1 might be used. For some reproductive and developmental effects, chronic exposure is that which covers a specific window of exposure that is relevant for eliciting the effect, and subchronic exposure would correspond to an exposure that is notably less than the full window of exposure.

Sometimes a database UF is also applied to address limitations or uncertainties in the database. The overall database for TCE is quite extensive, with studies for many different types of effects, including two-generation reproductive studies, as well as neurological and immunological studies. In addition, there were sufficient data to develop a reliable PBPK model to estimate route-to-route extrapolated doses for some candidate critical effects for which data were only available for one route of exposure. Thus, there is a high degree of confidence that the TCE database was sufficient to identify sensitive endpoints, and no database UF was used in this assessment.

6.2.1.2.1. Candidate Critical Effects and Reference Values (see Sections 5.1.2 and 5.1.3)

A large number of endpoints and studies were considered within each health effect domain. Chapter 5 contains a comprehensive discussion of all endpoints/studies that were considered for developing candidate reference values (cRfCs, cRfDs, p-cRfCs, and p-cRfDs), their PODs, and the UFs applied. The summary below reviews the selection of candidate critical effects for each health effect domain, the confidence in the reference values, the selection of PBPK model-based dose-metrics, and the impact of PBPK modeling on the candidate reference values.

6.2.1.2.2. Neurological effects

Candidate reference values were developed for several neurological domains for which there was evidence of hazard (see Tables 5-2 and 5-13). There is higher confidence in the candidate reference values for trigeminal nerve, auditory, or psychomotor effects, but the available data suggest that the more sensitive indicators of TCE neurotoxicity are changes in wakefulness, regeneration of the sciatic nerve, demyelination in the hippocampus, and degeneration of dopaminergic neurons. Therefore, these more sensitive effects are considered the candidate critical effects for neurotoxicity, albeit with more uncertainty in the corresponding candidate reference values. Of these more sensitive effects, there is greater confidence in the changes in wakefulness reported by Arito et al. (1994). In addition, trigeminal nerve effects are considered a candidate critical effect because this is the only type of neurological effect for which human data are available, and the POD for this effect is similar to that from the most sensitive rodent study (Arito et al., 1994, for changes in wakefulness). Between the two human studies of trigeminal nerve effects, Ruijten et al. (1991) is preferred for deriving noncancer reference values because its exposure characterization is considered more reliable.

Because of the lack of specific data as to the metabolites involved and the mode of action for the candidate critical neurologic effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) were selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. With these dose-metrics, the candidate reference values derived using the PBPK model were only modestly (~threefold or less) different than those derived on the basis of applied dose.

6.2.1.2.3. Kidney effects

Candidate reference values were developed for histopathological and weight changes in the kidney (see Tables 5-4 and 5-15), and these are considered to be candidate critical effects for several reasons. First, they appear to be the most sensitive indicators of toxicity that are available for the kidney. In addition, as discussed in Sections 3.3 and 3.5, both in vitro and in vivo pharmacokinetic data indicate substantially more production of GSH-conjugates thought to mediate TCE kidney effects in humans relative to rats and mice. Several studies are considered reliable for developing candidate reference values for these endpoints. For histopathological changes, these were the only available inhalation study (the rat study of Maltoni et al., 1986), the NTP (1988) study in rats, and the NCI (NCI, 1976) study in mice. For kidney weight changes, both available studies (Woolhiser et al., 2006; Kjellstrand et al., 1983a) were chosen as candidate critical studies.

Due to the substantial evidence supporting the role of GSH conjugation metabolites in TCE-induced nephrotoxicity, the preferred PBPK model dose-metrics for kidney effects were the amount of DCVC bioactivated in the kidney for rat studies and the amount of GSH conjugation (both scaled by body weight to the ¾ power) for mouse studies (inadequate toxicokinetic data are available in mice for predicting the amount of DCVC bioactivation). With these dose-metrics, the candidate reference values derived using the PBPK model were 300–400-fold lower than those derived on the basis of applied dose. As discussed above and in Chapter 3, this is due to the available in vivo and in vitro data supporting not only substantially more GSH conjugation in humans than in rodents, but also substantial interindividual toxicokinetic variability. Overall, there is high confidence in the nephrotoxic hazard from TCE exposure and in the appropriateness of the dose-metrics discussed above; however, there is substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data (see Section 3.3.3.2).

6.2.1.2.4. Liver effects

Hepatomegaly appears to be the most sensitive indicator of toxicity that is available for the liver and is therefore considered a candidate critical effect. Several studies are considered reliable for developing high-confidence candidate reference values for this endpoint. Since they all indicated similar sensitivity but represented different species and/or routes of exposure, they were all considered candidate critical studies (see Tables 5-4 and 5-14).

Due to the substantial evidence supporting the role of oxidative metabolism in TCE-induced hepatomegaly (and evidence against TCA being the sole mediator of TCE-induced hepatomegaly (Evans et al., 2009)), the preferred PBPK model dose-metric for liver effects was the amount of hepatic oxidative metabolism (scaled by body weight to the ¾ power). Total (hepatic and extrahepatic) oxidative metabolism (scaled by body weight to the ¾ power) was used as an alternative dose-metric. With these dose-metrics, the candidate reference values derived using the PBPK model were only modestly (~threefold or less) different than those derived on the basis of applied dose.

6.2.1.2.5. Immunological effects

There is high qualitative confidence for TCE immunotoxicity and moderate confidence in the candidate reference values that can be derived from the available studies (see Tables 5-6 and 5-16). Decreased thymus weight reported at relatively low exposures in nonautoimmune-prone mice is a clear indicator of immunotoxicity (Keil et al., 2009), and is therefore considered a candidate critical effect. A number of studies have also reported changes in markers of immunotoxicity at relatively low exposures. Among markers for autoimmune effects, the more sensitive measures of autoimmune changes in liver and spleen (Kaneko et al., 2000) and increased anti-dsDNA and anti-ssDNA antibodies (early markers for autoimmune disease) (Keil et al., 2009) are considered the candidate critical effects. For markers of immunosuppression, the more sensitive measures of decreased PFC response (Woolhiser et al., 2006), decreased stem cell bone marrow recolonization, and decreased cell-mediated response to sRBC (both from Sanders et al., 1982b) are considered the candidate critical effects. Developmental immunological effects are discussed below as part of the summary of developmental effects.

Because of the lack of specific data as to the metabolites involved and the mode of action for the candidate critical immunologic effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) was selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. With these dose-metrics, the candidate reference values derived using the PBPK model were, with one exception, only modestly (~threefold or less) different than those derived on the basis of applied dose. For the Woolhiser et al. (2006) decreased PFC response, with the alternative dose-metric of AUC of TCE in blood, BMD modeling based on internal doses changed the candidate reference value by 17-fold higher than the cRfC based on applied dose. However, the dose-response model fit for this effect using this metric was substantially worse than the fit using the preferred metric of total oxidative metabolism, with which the change in candidate reference value was only 1.3-fold.

6.2.1.2.6. Reproductive effects

While there is high qualitative confidence in the male reproductive hazard posed by TCE, there is lower confidence in the reference values that can be derived from the available studies of these effects (see Tables 5-8 and 5-17). Relatively high PODs are derived from several studies reporting less sensitive endpoints (George et al., 1986; George et al., 1985; Land et al., 1981), and correspondingly higher cRfCs and cRfDs suggest that they are not likely to be critical effects. The studies reporting more sensitive endpoints also tend to have greater uncertainty. For the human study by Chia et al. (1996), there are uncertainties in the characterization of exposure and the adversity of the effect measured in the study. For the Kumar et al. (2001b; 2000a; 2000b), Forkert et al. (2002), and Kan et al. (2007) studies, the severity of the sperm and testes effects appears to be continuing to increase with duration even at the end of the study, so it is plausible that a lower exposure for a longer duration may elicit similar effects. For the DuTeaux et al. (2004a) study, there is also duration- and low-dose extrapolation uncertainty due to the short duration of the study in comparison to the time period for sperm development as well as the lack of a NOAEL at the tested doses. Overall, even though there are limitations in the quantitative assessment, there remains sufficient evidence to consider these to be candidate critical effects.

There is moderate confidence both in the hazard and the candidate reference values for reproductive effects other than male reproductive effects. While there are multiple studies suggesting decreased maternal body weight with TCE exposure, this systemic change may not be indicative of more sensitive reproductive effects. None of the estimates developed from other reproductive effects is particularly uncertain or unreliable. Therefore, delayed parturition (Narotsky et al., 1995) and decreased mating (George et al., 1986), which yielded the lowest cRfDs, were considered candidate critical effects. These effects were also included so that candidate critical reproductive effects from oral studies would not include only that reported by DuTeaux et al. (2004a), from which deriving the cRfD entailed a higher degree of uncertainty.

Because of the general lack of specific data as to the metabolites involved and the mode of action for the candidate critical developmental effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) was selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. The only exception to this was for the DuTeaux et al. (2004a) study, which suggested that local oxidative metabolism of TCE in the male reproductive tract was involved in the effects reported. Therefore, in this case, AUC of TCE in blood was considered the preferred dose-metric, while total oxidative metabolism (scaled by body weight to the ¾ power) was considered the alternative metric. With these dose-metrics, the candidate reference values derived using the PBPK model were only modestly (~3.5-fold or less) different than those derived on the basis of applied dose.

6.2.1.2.7. Developmental effects

There is moderate-to-high confidence both in the hazard and the candidate reference values for developmental effects of TCE (see Tables 5-10 and 5-18). It is also noteworthy that the PODs for the more sensitive developmental effects were similar to or, in most cases, lower than the PODs for the more sensitive reproductive effects, suggesting that developmental effects are not a result of paternal or maternal toxicity. Among inhalation studies, candidate reference values were only developed for effects in rats reported in Healy et al. (1982), of resorptions, decreased fetal weight, and delayed skeletal ossification. These were all considered candidate critical developmental effects. Because resorptions were also reported in oral studies, the most sensitive (rat) oral study for this effect (and most reliable for dose-response analysis) of Narotsky et al. (1995) was also selected as a candidate critical study. The confidence in the oral studies and candidate reference values developed for more sensitive endpoints is more moderate, but still sufficient for consideration as candidate critical effects. The most sensitive endpoints by far are the increased fetal heart malformations in rats reported by Johnson et al. (2003) and the developmental immunotoxicity in mice reported by Peden-Adams et al. (2006), and these are both considered candidate critical effects. Neurodevelopmental effects are a distinct type among developmental effects. Thus, the next most sensitive endpoints of decreased rearing postexposure in mice (Fredriksson et al., 1993), increased exploration postexposure in rats (Taylor et al., 1985), and decreased myelination in the hippocampus of rats (Isaacson and Taylor, 1989) are also considered candidate critical effects.

Because of the general lack of specific data as to the metabolites involved and the mode of action for the candidate critical developmental effects, PBPK model predictions of total metabolism (scaled by body weight to the ¾ power) was selected as the preferred dose-metric based on the general observation that TCE toxicity is associated with metabolism. The AUC of TCE in blood was used as an alternative dose-metric. The only exception to this was for the Johnson et al. (2003) study, which suggested that oxidative metabolites were involved in the effects reported based on similar effects being reported from TCA and DCA exposure. Therefore, in this case, total oxidative metabolism (scaled by body weight to the ¾ power) was considered the preferred dose-metric, while AUC of TCE in blood was considered the alternative metric. With these dose-metrics, the candidate reference values derived using the PBPK model were, with one exception, only modestly (~threefold or less) different than those derived on the basis of applied dose. For resorptions reported by Narotsky et al. (1995), BMD modeling based on internal doses changed the candidate reference value by seven to eightfold larger than the corresponding cRfD based on applied dose. However, there is substantial uncertainty in the low-dose curvature of the dose-response curve for modeling both with applied and internal dose, so the BMD remains somewhat uncertain for this endpoint/study. Finally, for two studies (Peden-Adams et al., 2006; Isaacson and Taylor, 1989), PBPK modeling of internal doses was not performed due to the inability to model the complicated exposure pattern (in utero, followed by lactational transfer, followed by drinking water postweaning).

6.2.1.2.8. Summary of most sensitive candidate reference values

As shown in Sections 5.1.3 and 5.1.5, the most sensitive candidate reference values are for the developmental effect of heart malformations in rats (candidate RfC of 0.0004 ppm and candidate RfD of 0.0005 mg/kg/day), developmental immunotoxicity in mice exposed pre- and postnatally (candidate RfD of 0.0004 mg/kg/day), immunological effects in mice (lowest candidate RfCs of 0.0003–0.003 ppm and lowest candidate RfDs of 0.0005–0.005 mg/kg/day), and kidney effects in rats and mice (candidate RfCs of 0.0006–0.002 ppm and candidate RfDs of 0.0003–0.001 mg/kg/day). The most sensitive candidate reference values also generally have low composite UFs (with the exception of some mouse immunological and kidney effects), so they are expected to be reflective of the most sensitive effects as well. Thus, the most sensitive candidate references values for multiple effects span about an order of magnitude for both inhalation (0.0003–0.003 ppm [0.002–0.02 mg/m^3]) and oral (0.0004–0.005 mg/kg/day) exposures. The most sensitive candidate references values for neurological and reproductive effects are about an order of magnitude higher (lowest candidate RfCs of 0.007–0.02 ppm [0.04–0.1 mg/m^3] and lowest candidate RfDs of 0.009–0.02 mg/kg/day). Lastly, the liver effects have candidate reference values that are another two orders of magnitude higher (candidate RfCs of 1–2 ppm [6–10 mg/m^3] and candidate RfDs of 0.9–2 mg/kg/day).

6.2.1.3. Noncancer Reference Values (see Section 5.1.5)

6.2.1.3.1. RfC

The goal is to select an overall RfC that is well supported by the available data (i.e., without excessive uncertainty given the extensive database) and protective for all of the candidate critical effects, recognizing that individual candidate RfC values are by nature somewhat imprecise. As discussed in Section 5.1, the lowest candidate RfC values within each health effect category span a 3,000-fold range from 0.0003 to 0.9 ppm (see Table 5-26). One approach to selecting an RfC would be to select the lowest calculated value of 0.0003 ppm for decreased thymus weight in mice. However, three candidate RfCs (cRfCs and p-cRfCs) are in the relatively narrow range of 0.0003–0.0006 ppm at the low end of the overall range (see Table 5-24). Given the somewhat imprecise nature of the individual candidate RfC values, and the fact that multiple effects/studies lead to similar candidate RfC values, the approach taken in this assessment is to select an RfC supported by multiple effects/studies. The advantages of this approach, which is only possible when there is a relatively large database of studies/effects and when multiple candidate values happen to fall within a narrow range at the low end of the overall range, are that it leads to a more robust RfC (less sensitive to limitations of individual studies) and that it provides the important characterization that the RfC exposure level is similar for multiple noncancer effects rather than being based on a sole explicit critical effect.

Therefore, two critical and one supporting studies/effects were chosen as the basis of the RfC for TCE noncancer effects (see Tables 5-28 and 5-29). These lowest candidate RfCs, ranging from 0.0003 to 0.0006 ppm for developmental, kidney, and immunologic effects, are values derived from route-to-route extrapolation using the PBPK model. The lowest candidate RfC estimate from an inhalation study is 0.001 ppm for kidney effects, which is higher than the route-to-route extrapolated candidate RfC estimate from the most sensitive oral study. For all of the candidate RfCs, the PBPK model was used for inter- and intraspecies extrapolation, based on the preferred dose-metric for each endpoint. There is moderate-to-high confidence in the lowest candidate RfC for immunological effects (see Section 5.1.2.5), and moderate confidence in the lowest candidate RfC for developmental effects (heart malformations) (see Section 5.1.2.8); these are considered the critical effects for deriving the RfC. For kidney effects (toxic nephropathy), there is high confidence in the nephrotoxic hazard from TCE exposure and in the appropriateness of the selected dose-metric; however, as discussed in Section 3.3.3.2, there remains substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data, and thus toxic nephropathy is considered a supporting effect.

As a whole, the estimates support an RfC of 0.0004 ppm (0.4 ppb or 2 $\mu g/m^3$). This value essentially reflects the midpoint between the similar candidate RfC estimates for the two critical effects (0.00033 ppm for decreased thymus weight in mice and 0.00037 ppm for heart malformations in rats), rounded to one significant figure. This value is also within a factor of 2 of the candidate RfC estimate of 0.0006 ppm for the supporting effect of toxic nephropathy in rats. Thus, this assessment does not rely on a single estimate alone; rather, each estimate is supported by estimates of similar magnitude from other effects. In other words, there is robust support for an RfC of 0.0004 ppm provided by estimates for multiple effects from multiple studies. The estimates are based on PBPK model-based estimates of internal dose for interspecies, intraspecies, and route-to-route extrapolation, and there is sufficient confidence in the PBPK model and support from mechanistic data for one of the dose-metrics (total oxidative metabolism for the heart malformations). There is high confidence that bioactivation of DCVC and total GSH metabolism would be appropriate dose-metrics for toxic nephropathy, but there is substantial uncertainty in the PBPK model predictions for these dose-metrics in humans (see Section 5.1.3.1). Note that there is some human evidence of developmental heart defects from TCE exposure in community studies (see Section 4.8.3.1.1) and of kidney toxicity in TCE-exposed workers (see Section 4.4.1).

In summary, the RfC is **0.0004 ppm** (0.4 ppb or 2 $\mu g/m^3$) based on route-to-route extrapolated results from oral studies for the critical effects of heart malformations (rats) and immunotoxicity (mice). This RfC value is further supported by route-to-route extrapolated results from an oral study of toxic nephropathy (rats).

6.2.1.3.2. RfD

As with the RfC determination above, the goal is to select an overall RfD that is well-supported by the available data (i.e., without excessive uncertainty given the extensive database) and protective for all of the candidate critical effects, recognizing that individual candidate RfD values are by nature somewhat imprecise. As discussed in Section 5.1, the lowest candidate RfD values (cRfDs and p-cRfDs) within each health effect category span a nearly 3,000-fold range from 0.0003 to 0.8 mg/kg/day (see Table 5-26). However, multiple candidate RfDs are in the relatively narrow range of 0.0003–0.0008 mg/kg/day at the low end of the overall range. Given the somewhat imprecise nature of the individual candidate RfD values, and the fact that multiple effects/studies lead to similar candidate RfD values, the approach taken in this assessment is to select an RfD supported by multiple effects/studies. The advantages of this approach, which is only possible when there is a relatively large database of studies/effects and when multiple candidate values happen to fall within a narrow range at the low end of the overall range, are that it leads to a more robust RfD (less sensitive to limitations of individual studies) and that it provides the important characterization that the RfD exposure level is similar for multiple noncancer effects rather than being based on a sole explicit critical effect.

Therefore, three critical and two supporting studies/effects were chosen as the basis of the RfD for TCE noncancer effects (see Tables 5-30 and 5-31). All but one of the lowest candidate RfD values—0.0008 mg/kg/day for increased kidney weight in rats, 0.0005 mg/kg/day for both heart malformations in rats and decreased thymus weights in mice, and 0.0003 mg/kg/day for increased toxic nephropathy in rats—are derived using the PBPK model for inter- and intraspecies extrapolation, based on the preferred dose-metric for each endpoint, and the latter value is derived also using the PBPK model for route-to-route extrapolation from an inhalation study. The other of these lowest candidate RfDs—0.0004 mg/kg/day for developmental immunotoxicity (decreased PFC response and increased delayed-type hypersensitivity) in mice—is based on applied dose. There is moderate-to-high confidence in the candidate RfDs for decreased thymus weights (see Section 5.1.2.5) and developmental immunological effects, and moderate confidence in that for heart malformations (see Section 5.1.2.8); these are considered the critical effects for deriving the RfC. For kidney effects, there is high confidence in the nephrotoxic hazard from TCE exposure and in the appropriateness of the selected dose-metric; however, as discussed in Section 3.3.3.2, there remains substantial uncertainty in the extrapolation of GSH conjugation from rodents to humans due to limitations in the available data, and thus these effects are considered supporting effects.

As a whole, the estimates support an RfD of 0.0005 mg/kg/day. This value is within 20% of the estimates for the critical effects—0.0004 mg/kg/day for developmental immunotoxicity (decreased PFC and increased delayed-type hypersensitivity) in mice and 0.0005 mg/kg/day for both heart malformations in rats and decreased thymus weights in mice. This value is also within approximately a factor of 2 of the supporting effect estimates of 0.0003 mg/kg/day for toxic nephropathy in rats and 0.0008 mg/kg/day for increased kidney weight in rats. Thus, this assessment does not rely on any single estimate alone; rather, each estimate is supported by estimates of similar magnitude from other effects. In other words, there is strong, robust support for an RfD of 0.0005 mg/kg/day provided by the concordance of estimates derived from multiple effects from multiple studies. The estimates for kidney effects, thymus effects, and developmental heart malformations are based on PBPK model-based estimates of internal dose for interspecies and intraspecies extrapolation, and there is sufficient confidence in the PBPK model and support from mechanistic data for one of the dose-metrics (total oxidative metabolism for the heart malformations). There is high confidence that bioactivation of DCVC would be an appropriate dose-metric for toxic nephropathy, but there is substantial uncertainty in the PBPK model predictions for this dose-metric in humans (see Section 5.1.3.1). Note that there is some human evidence of developmental heart defects from TCE exposure in community studies (see Section 4.8.3.1.1) and of kidney toxicity in TCE-exposed workers (see Section 4.4.1).

In summary, the RfD is **0.0005 mg/kg/day** based on the critical effects of heart malformations (rats), adult immunological effects (mice), and developmental immunotoxicity (mice), and toxic nephropathy (rats), all from oral studies. This RfD value is further supported by results from an oral study for the effect of toxic nephropathy (rats) and route-to-route extrapolated results from an inhalation study for the effect of increased kidney weight (rats).

6.2.2. Cancer (see Section 5.2)

6.2.2.1. Background and Methods (rodent: see Section 5.2.1.1; human: see Section 5.2.2.1)

As summarized above, following EPA (2005b) *Guidelines for Carcinogen Risk Assessment*, TCE is characterized as “carcinogenic to humans” by all routes of exposure, based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer, but there is also human evidence of TCE carcinogenicity in the liver and lymphoid tissues. This conclusion is further supported by rodent bioassay data indicating carcinogenicity of TCE in rats and mice at tumor sites that include those identified in human epidemiologic studies. Therefore, both human epidemiologic studies as well as rodent bioassays were considered for deriving PODs for dose-response assessment of cancer endpoints. For PODs derived from rodent bioassays, default dosimetry procedures were applied to convert applied rodent doses to HEDs. Essentially, for inhalation exposures, "ppm equivalence" across species was assumed, as recommended by U.S. EPA (1994a) for Category 3 gases for which the blood:air partition coefficient in laboratory animals is greater than that in humans. For oral doses, ¾-power body-weight scaling was used, with a default average human body weight of 70 kg. In addition to applied doses, several internal dose-metrics estimated using a PBPK model for TCE and its metabolites were used in the dose-response modeling for each tumor type. In general, an attempt was made to use tissue-specific dose-metrics representing particular pathways or metabolites identified from available data as having a likely role in the induction of a tissue-specific cancer. Where insufficient information was available to establish particular metabolites or pathways of likely relevance to a tissue-specific cancer, more general "upstream" metrics had to be used. In addition, the selection of dose-metrics was limited to metrics that could be adequately estimated by the PBPK model.

Regarding low-dose extrapolation, a key consideration in determining what extrapolation approach to use is the mode(s) of action. However, mode-of-action data are lacking or limited for each of the cancer responses associated with TCE exposure, with the exception of the kidney tumors. For the kidney tumors, the weight of the available evidence supports the conclusion that a mutagenic mode of action is operative; this mode of action supports linear low-dose extrapolation. The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking TCE-induced proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Moreover, it is unlikely that any contribution from cytotoxicity leads to a non-linear dose-response relationship near the PODs. In the case of the rodent bioassays, maximal levels of toxicity are reached before the onset of tumors. Finally, because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the dose-response relationship would nonetheless be expected to be linear at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against the use of linear extrapolation from the POD. For the other TCE-induced cancers, the mode(s) of action is unknown. When the mode(s) of action cannot be clearly defined, EPA generally uses a linear approach to estimate low-dose risk (2005b), based on the following general principles:

- A chemical's carcinogenic effects may act additively to ongoing biological processes, given that diverse human populations are already exposed to other agents and have substantial background incidences of various cancers.
- A broadening of the dose-response curve (i.e., less rapid fall-off of response with decreasing dose) in diverse human populations and, accordingly, a greater potential for

risks from low-dose exposures (Lutz et al., 2005; Zeise et al., 1987) is expected for two reasons. First, even if there is a "threshold" concentration for effects at the cellular level, that threshold is expected to differ across individuals. Second, greater variability in response to exposures would be anticipated in heterogeneous populations than in inbred laboratory species under controlled conditions (due to, e.g., genetic variability, disease status, age, nutrition, and smoking status).

- The general use of linear extrapolation provides reasonable upper-bound estimates that are believed to be health-protective (U.S. EPA, 2005b) and also provides consistency across assessments.

6.2.2.2. Inhalation Unit Risk Estimate (rodent: see Section 5.2.1.3; human: see Sections 5.2.2.1 and 5.2.2.2)

The inhalation unit risk for TCE is defined as a plausible upper bound lifetime extra risk of cancer from chronic inhalation of TCE per unit of air concentration. The inhalation unit risk for TCE is 2.20×10^{-2} per ppm (**2×10^{-2} per ppm [4×10^{-6} per μg/m^3]** rounded to one significant figure), based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer. This estimate is based on good-quality human data, thus avoiding the uncertainties inherent in interspecies extrapolation. The Charbotel et al. (2006) case-control study of 86 incident RCC cases and 316 age- and sex-matched controls, with individual cumulative exposure estimates for TCE inhalation for each subject, provides a sufficient human data set for deriving quantitative cancer risk estimates for RCC in humans. The study is a high-quality study that used a detailed exposure assessment (Fevotte et al., 2006) and took numerous potential confounding factors, including exposure to other chemicals, into account. A significant dose-response relationship was reported for cumulative TCE exposure and RCC (Charbotel et al., 2006). Human data on TCE exposure and cancer risk sufficient for dose-response modeling are only available for RCC, yet human and rodent data suggest that TCE exposure increases the risk of other cancers as well. In particular, there is evidence from human (and rodent) studies for increased risks of lymphoma and liver cancer. Therefore, the inhalation unit risk estimate derived from human data for RCC incidence was adjusted to account for potential increased risk of those cancer types. To make this adjustment, a factor accounting for the relative contributions to the extra risk for cancer incidence from TCE exposure for these three cancer types combined versus the extra risk for RCC alone was estimated, and this factor was applied to the unit risk estimate for RCC to obtain a unit risk estimate for the three cancer types combined (i.e., lifetime extra risk for developing *any* of the three types of cancer). This estimate is considered a better estimate of total cancer risk from TCE exposure than the estimate for RCC alone. Although only the Charbotel et al. (2006) study was found adequate for direct estimation of inhalation unit risks, the available epidemiologic data provide sufficient information for estimating the *relative* potency of TCE across tumor sites. In particular, the relative contributions to extra risk (for cancer incidence) were calculated from two different data sets to derive the adjustment factor for adjusting the unit risk estimate for RCC to a unit risk estimate for the three types of cancers (RCC, NHL, and liver) combined. The first calculation is based on the results of the meta-analyses of human epidemiologic data for the three cancer types; the second calculation is based on the results of the Raaschou-Nielsen et al. (2003) study, the largest single human epidemiologic study by far with RR estimates for all three cancer types. These calculations support an adjustment factor of 4.

The inhalation unit risk based on human epidemiologic data is supported by inhalation unit risk estimates from multiple rodent bioassays, the most sensitive of which range from **1×10^{-2} to 2×10^{-1} per ppm [2×10^{-6} to 3×10^{-5} per μg/m^3]**. From the inhalation bioassays selected for analysis in Section 5.2.1.1, and using the preferred PBPK model-based dose-metrics, the inhalation unit risk estimate for the most sensitive sex/species is 8×10^{-2} per ppm [2×10^{-5} per μg/m^3], based on kidney adenomas and carcinomas reported by Maltoni et al. (1986) for male Sprague-Dawley rats. Leukemias and Leydig cell tumors were also increased in these rats, and, although a combined analysis for these cancer types which incorporated the different site-specific preferred dose-metrics was not performed, the result of such an analysis is expected to be similar, about 9×10^{-2} per ppm [2×10^{-5} per μg/m^3]. The next most sensitive sex/species from the inhalation bioassays is the female mouse, for which lymphomas were reported by Henschler et al. (1980); these data yield a unit risk estimate of 1.0×10^{-2} per ppm [2×10^{-6} per μg/m^3]. In addition, the 90% CIs (i.e., 5–95% bounds) reported in Table 5-41 for male rat kidney tumors from Maltoni et al. (1986) and female mouse lymphomas from Henschler et al. (1980), derived from the quantitative analysis of PBPK model uncertainty, both included the estimate based on human data of 2×10^{-2} per ppm. Furthermore, PBPK model-based route-to-route extrapolation of the results for the most sensitive sex/species from the oral bioassays, kidney tumors in male Osborne-Mendel rats and testicular tumors in Marshall rats (NTP, 1988), leads to inhalation unit risk estimates of 2×10^{-1} per ppm [3×10^{-5} per μg/m^3] and 4×10^{-2} per ppm [8×10^{-6} per μg/m^3], respectively, with the preferred estimate based on human data falling within the route-to-route extrapolation of the 90% CIs reported in Table 5-42. Finally, for all of these estimates, the ratios of BMDs to the BMDLs did not exceed a value of 3, indicating that the uncertainties in the dose-response modeling for determining the POD in the observable range are small.

Although there are uncertainties in these various estimates, confidence in the proposed inhalation unit risk estimate of 2×10^{-2} per ppm [4×10^{-6} per μg/m^3], based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer (as summarized in Section 6.1.4), is further increased by the similarity of this estimate to estimates based on multiple rodent data sets. Application of the ADAFs for the kidney cancer risks, due to the weight of evidence supporting a mutagenic mode of action for this endpoint, is summarized in Section 6.2.2.5.

6.2.2.3. Oral Slope Factor Estimate (rodent: see Section 5.2.1.3; human: see Section 5.2.2.3)

The oral slope factor for TCE is defined as a plausible upper bound lifetime extra risk of cancer from chronic ingestion of TCE per mg/kg/day oral dose. The oral slope factor is 4.64×10^{-2} per mg/kg/day (**5×10^{-2} per mg/kg/day** rounded to one significant figure), resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer. This estimate is based on good-quality human data, thus avoiding uncertainties inherent in interspecies extrapolation. In addition, uncertainty in the PBPK model-based route-to-route extrapolation is relatively low (Chiu, 2006; Chiu and White, 2006). In this particular case, extrapolation using different dose-metrics yielded expected population mean risks within about a twofold range, and, for any particular dose-metric, the 95% CI for the extrapolated population mean risks for each site spanned a range of no more than about threefold.

This value is supported by oral slope factor estimates from multiple rodent bioassays, the most sensitive of which range from **3×10^{-2} to 3×10^{-1} per mg/kg/day**. From the oral bioassays selected for analysis in Section 5.2.1.1, and using the preferred PBPK model-based dose-metrics, the oral slope factor estimate for the most sensitive sex/species is 3×10^{-1} per mg/kg/day, based on kidney tumors in male Osborne-Mendel rats (NTP, 1988). The oral slope factor estimate for testicular tumors in male Marshall rats (NTP, 1988) is somewhat lower at 7×10^{-2} per mg/kg/day. The next most sensitive sex/species result from the oral studies is for male mouse liver tumors (NCI, 1976), with an oral slope factor estimate of 3×10^{-2} per mg/kg/day. In addition, the 90% CIs reported in Table 5-42 for male Osborne-Mendel rat kidney tumors (NTP, 1988), male F344 rat kidney tumors (NTP, 1990), and male Marshall rat testicular tumors (NTP, 1988), derived from the quantitative analysis of PBPK model uncertainty, all included the estimate based on human data of 5×10^{-2} per mg/kg/day, while the upper 95% confidence bound for male mouse liver tumors from NCI (1976) was slightly below this value at 4×10^{-2} per mg/kg/day. Furthermore, PBPK model-based route-to-route extrapolation of the most sensitive endpoint from the inhalation bioassays, male rat kidney tumors from Maltoni et al. (1986), leads to an oral slope factor estimate of 1×10^{-1} per mg/kg/day, with the preferred estimate based on human data falling within the route-to-route extrapolation of the 90% CI reported in Table 5-41. Finally, for all of these estimates, the ratios of BMDs to the BMDLs did not exceed a value of 3, indicating that the uncertainties in the dose-response modeling for determining the POD in the observable range are small.

Although there are uncertainties in these various estimates, confidence in the proposed oral slope factor estimate of 5×10^{-2} per mg/kg/day, resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted for potential risk for NHL and liver cancer (as summarized above for the inhalation unit risk estimate, but with an adjustment factor of 5 for oral exposure because of the differences in the relative values of the dose-metrics), is further increased by the similarity of this estimate to estimates based on multiple rodent data sets. Application of the ADAFs for the kidney cancer risks, due to the weight of evidence supporting a mutagenic mode of action for this endpoint, is summarized below in Section 6.2.2.5.

6.2.2.4. Uncertainties in Cancer Dose-Response Assessment

6.2.2.4.1. Uncertainties in estimates based on human epidemiologic data (see Section 5.2.2.1.3)

All risk assessments involve uncertainty, as study data are extrapolated to make general inferences about potential effects in humans from environmental exposure. The values for the slope factor and unit risk estimates are based on good quality human data, which avoids interspecies extrapolation, one of the major sources of uncertainty in quantitative cancer risk estimates.

A remaining major uncertainty in the unit risk estimate for RCC incidence derived from the Charbotel et al. (2006) study is the extrapolation from occupational exposures to lower environmental exposures. There was some evidence of a contribution to increased RCC risk from peak exposures; however, there remained an apparent dose-response relationship for RCC risk with increasing cumulative exposure without peaks, and the OR for exposure with peaks compared to exposure without peaks was not significantly elevated (Charbotel et al., 2006). Although the actual exposure-response relationship at low exposure levels is unknown, the conclusion that a mutagenic mode of action is operative for TCE-induced kidney tumors supports the linear low-dose extrapolation that was used (U.S. EPA, 2005b). Additional support for use of linear extrapolation is discussed above in Section 6.2.2.1.

Another source of uncertainty is the dose-response model used to model the study data to estimate the POD. A weighted linear regression across the categorical ORs was used to obtain a slope estimate; use of a linear model in the observable range of the data is often a good general approach for human data because epidemiological data are frequently too limited (the Charbotel et al. [(2006)] study had 86 RCC cases, 37 of which had TCE exposure) to clearly identify an alternate model (U.S. EPA, 2005b). The ratio of the maximum likelihood estimate of the effective concentration for a 1% response (EC_{01}) to the LEC_{01}, which gives some indication of the statistical uncertainties in the dose-response modeling, was about a factor of 2.

A further source of uncertainty is the retrospective estimation of TCE exposures in the Charbotel et al. (2006) study. This case-control study was conducted in the Arve Valley in France, a region with a high concentration of screw cutting workshops using TCE and other degreasing agents. Since the 1960s, occupational physicians of the region have collected a large quantity of well-documented measurements, including TCE air concentrations and urinary metabolite levels (Fevotte et al., 2006). The study investigators conducted a comprehensive exposure assessment to estimate cumulative TCE exposures for the individual study subjects, using a detailed occupational questionnaire with a customized task-exposure matrix for the screw-cutting workers and a more general occupational questionnaire for workers exposed to TCE in other industries (Fevotte et al., 2006). The exposure assessment also attempted to take dermal exposure from hand-dipping practices into account by equating it with an equivalent airborne concentration based on biological monitoring data. Despite the appreciable effort of the investigators, considerable uncertainty associated with any retrospective exposure assessment is inevitable, and some exposure misclassification is unavoidable. Such exposure misclassification was most likely for the 19 deceased cases and their matched controls, for which proxy respondents were used, and for exposures outside the screw-cutting industry. The exposure estimates from the RCC study of Moore et al. (2010) were not considered to be as quantitatively accurate as those of Charbotel et al. (2006) and so were not used for derivation of a unit risk estimate (see Section 5.2.2); nonetheless, it should be noted that these exposure estimates are substantially lower than those of Charbotel et al. (2006) for comparable OR estimates. If the exposure estimates for Charbotel et al. (2006) are overestimated, as suggested by the exposure estimates from Moore et al. (2010), the slope of the linear regression model, and hence the unit risk estimate, would be correspondingly underestimated.

Another source of uncertainty in the Charbotel et al. (2006) study is the possible influence of potential confounding or modifying factors. This study population, with a high prevalence of metal-working, also had relatively high prevalences of exposure to petroleum oils, cadmium, petroleum solvents, welding fumes, and asbestos (Fevotte et al., 2006). Other exposures assessed included other solvents (including other chlorinated solvents), lead, and ionizing radiation. None of these exposures was found to be significantly associated with RCC at a $p = 0.05$ significance level. Cutting fluids and other petroleum oils were associated with RCC at a $p = 0.1$ significance level; however, further modeling suggested no association with RCC when other significant factors were taken into account (Charbotel et al., 2006). Moreover, a review of other studies suggested that potential confounding from cutting fluids and other petroleum oils is of minimal concern (see Section 4.4.2.3). Nonetheless, a sensitivity analysis was conducted using the OR estimates further adjusted for cutting fluids and other petroleum oils from the unpublished report by Charbotel et al. (2005), and an essentially identical unit risk estimate of 5.46×10^{-3} per ppm was obtained. In addition, the medical questionnaire included familial kidney disease and medical history, such as kidney stones, infection, chronic dialysis, hypertension, and use of antihypertensive drugs, diuretics, and analgesics. BMI was also calculated, and lifestyle information such as smoking habits and coffee consumption was collected. Univariate analyses found high levels of smoking and BMI to be associated with increased odds of RCC, and these two variables were included in the conditional logistic regressions. Thus, although impacts of other factors are possible, this study took great pains to attempt to account for potential confounding or modifying factors.

Some other sources of uncertainty associated with the epidemiological data are the dose-metric and lag period. As discussed above, there was some evidence of a contribution to increased RCC risk from peak TCE exposures; however, there appeared to be an independent effect of cumulative exposure without peaks. Cumulative exposure is considered a good measure of total exposure because it integrates exposure (levels) over time. If there is a contributing effect of peak exposures, not already taken into account in the cumulative exposure metric, the linear slope may be overestimated to some extent. Sometimes, cancer data are modeled with the inclusion of a lag period to discount more recent exposures not likely to have contributed to the onset of cancer. In an unpublished report, Charbotel et al. (2005) also presented the results of a conditional logistic regression with a 10-year lag period, and these results are very similar to the unlagged results reported in their published paper, suggesting that the lag period might not be an important factor in this study.

Some additional sources of uncertainty are not so much inherent in the exposure-response modeling or in the epidemiologic data themselves but, rather, arise in the process of obtaining more general Agency risk estimates from the epidemiologic results. EPA cancer risk estimates are typically derived to represent an upper bound on increased risk of cancer incidence for all sites affected by an agent for the general population. From experimental animal studies, this is accomplished by using tumor incidence data and summing across all of the tumor sites that demonstrate significantly increased incidences, customarily for the most sensitive sex and species, to attempt to be protective of the general human population. However, in estimating comparable risks from the Charbotel et al. (2006) epidemiologic data, certain limitations are encountered. For one thing, these epidemiology data represent a geographically limited (Arve Valley, France) and likely not very diverse population of working adults. Thus, there is uncertainty about the applicability of the results to a more diverse general population.

Additionally, the Charbotel et al. (2006) study was a study of RCC only, and so the risk estimate derived from it does not represent all of the tumor sites that may be affected by TCE.

This uncertainty was addressed by adjusting the RCC estimate to multiple sites, but there are also uncertainties related to the assumptions inherent in the calculations for this adjustment. As discussed in Section 5.2.2.2, adequate quantitative dose-response data were only available for one cancer type in humans, so other human data were used to adjust the estimate derived for RCC to include risk for other cancers with substantial human evidence of hazard (NHL and liver cancer). The relative contributions to extra risk (for cancer incidence) were calculated from two different data sets to derive an adjustment factor. The first calculation is based on the results of the meta-analyses for the three cancer types; the second calculation is based on the results of the Raaschou-Nielsen et al. (2003) study, the largest single study by far with RR estimates for all three cancer types. The fact that the calculations based on two different data sets yielded comparable values for the adjustment factor (both within 25% of the selected factor of 4) provides more robust support for the use of the factor of 4. Additional uncertainties pertain to the weight of evidence supporting the association of TCE exposure with increased risk of cancer for the three cancer types. As discussed in Section 4.11.2, it is concluded that the weight of evidence for kidney cancer is sufficient to classify TCE as "carcinogenic to humans." It is also concluded that there is strong evidence that TCE causes NHL as well, although the evidence for liver cancer was more limited. In addition, the rodent studies demonstrate clear evidence of multisite carcinogenicity, with cancer types including those for which associations with TCE exposure are observed in human studies (i.e., liver and kidney cancers and lymphomas). Overall, the evidence is sufficiently persuasive to support the use of the adjustment factor of 4 based on these three cancer types. Alternatively, if one were to use the factor based only on the two cancer types with the strongest human evidence, the cancer inhalation unit risk estimate would be only slightly reduced (25%).

Finally, the value for the oral slope factor estimate was based on route-to-route extrapolation of the inhalation unit risk based on human data using predictions from the PBPK model. Because different internal dose-metrics are preferred for each target tissue site, a separate route-to-route extrapolation was performed for each site-specific slope factor estimate. As discussed above, uncertainty in the PBPK model-based route-to-route extrapolation is relatively low (Chiu, 2006; Chiu and White, 2006). In this particular case, extrapolation using different dose-metrics yielded expected population mean risks within about a twofold range, and, for any particular dose-metric, the 95% CI for the extrapolated population mean risks for each site spanned a range of no more than about threefold.

6.2.2.4.2. Uncertainties in estimates based on rodent bioassays (see Section 5.2.1.4)

With respect to rodent-based cancer risk estimates, the cancer risk is typically estimated from the total cancer burden from all sites that demonstrate an increased tumor incidence for the most sensitive experimental species and sex. It is expected that this approach is protective of the human population, which is more diverse but is exposed to lower exposure levels. In the case of TCE, the impact of selection of the bioassay is limited, since, as discussed in Sections 5.2.1.3 and 5.2.3, estimates based on the two or three most sensitive bioassays are within an order of magnitude of each other, and are consistent across routes of exposure when extrapolated using the PBPK model.

Another source of uncertainty in the TCE rodent-based cancer risk estimates is interspecies extrapolation. Several plausible PBPK model-based dose-metrics were used for extrapolation of toxicokinetics, but the cancer slope factor and unit risk estimates obtained using the preferred dose-metrics were generally similar (within about threefold) to those derived using default dosimetry assumptions, with the exception of the bioactivated DCVC dose-metric for rat kidney tumors and the metric for the amount of TCE oxidized in the respiratory tract for mouse lung tumors occurring from oral exposure. However, there is greater biological support for these selected dose-metrics. The uncertainty in the PBPK model predictions themselves was analyzed quantitatively through an analysis of the impact of parameter uncertainties in the PBPK model. The 95% lower bounds on the BMD including parameter uncertainties in the PBPK model were no more than fourfold lower than those based on central estimates of the PBPK model predictions. The greatest uncertainty was for slope factors and unit risks derived from rat kidney tumors, primarily reflecting the substantial uncertainty in the rat internal dose and in the extrapolation of GSH conjugation from rodents to humans.

Regarding low-dose extrapolation, a key consideration in determining what extrapolation approach to use is the mode(s) of action. However, mode-of-action data are lacking or limited for each of the cancer responses associated with TCE exposure, with the exception of the kidney tumors. For the kidney tumors, the weight of the available evidence supports the conclusion that a mutagenic mode of action is operative; this mode of action supports linear low-dose extrapolation. For the other TCE-induced cancers, the data either support a complex mode of action or are inadequate to specify the key events and modes of action involved. When the mode(s) of action cannot be clearly defined, EPA generally uses a linear approach to estimate low-dose risk (U.S. EPA, 2005b), based on the general principles discussed above.

With respect to uncertainties in the dose-response modeling, the two-step approach of modeling only in the observable range, as put forth in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), is designed in part to minimize model dependence. The ratios of the BMDs to the BMDLs, which give some indication of the statistical uncertainties in the dose-response modeling, did not exceed a value of 2.5 for all of the primary analyses used in this assessment. Thus, overall, modeling uncertainties in the observable range are considered to be minimal. Some additional uncertainty is conveyed by uncertainties in the survival adjustments made to some of the bioassay data; however, a comparison of the results of two different survival adjustment methods suggest that their impact is minimal relative to the uncertainties already discussed.

6.2.2.5. Application of ADAFs (see Section 5.2.3.3)

When there is sufficient weight of evidence to conclude that a carcinogen operates through a mutagenic mode of action, and in the absence of chemical-specific data on age-specific susceptibility, EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) recommends the application of default ADAFs to adjust for potential increased susceptibility from early-life exposure. See the *Supplemental Guidance* for detailed information on the general application of these adjustment factors. In brief, the *Supplemental Guidance* establishes ADAFs for three specific age groups. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2–<16 years, and 1 for ≥16 years (U.S. EPA, 2005b). For risk assessments based on specific exposure assessments, the 10- and 3-fold adjustments to the slope factor or unit risk estimates are to be combined with age-specific exposure estimates when estimating cancer risks from early-life (<16 years age) exposure.

In the case of TCE, the inhalation unit risk and oral slope factor estimates reflect lifetime risk for cancer at multiple sites, and a mutagenic mode of action has been established for one of these sites, the kidney. The weight of evidence also supports involvement of processes of cytotoxicity and regenerative proliferation in the carcinogenicity of TCE, although not with the extent of support as for a mutagenic mode of action. In particular, data linking TCE-induced proliferation to increased mutation or clonal expansion are lacking, as are data informing the quantitative contribution of cytotoxicity. Because any possible involvement of a cytotoxicity mode of action would be additional to mutagenicity, the mutagenic mode of action would be expected to dominate at low doses. Therefore, the additional involvement of a cytotoxicity mode of action does not provide evidence against application of ADAFs. In addition, as discussed in Section 4.10, inadequate TCE-specific data exists to quantify early-life susceptibility to TCE carcinogenicity; therefore, as recommended in the *Supplemental Guidance*, the default ADAFs are used. As illustrated in the example calculations in Sections 5.2.3.3.1 and 5.2.3.3.2, application of the default ADAFs to the kidney cancer inhalation unit risk and oral slope factor estimates for TCE is likely to have minimal impact on the total cancer risk except when exposure is primarily during early life.

In addition to the uncertainties discussed above for the inhalation and oral total cancer unit risk and slope factor estimates, there are uncertainties in the application of ADAFs to adjust for potential increased early-life susceptibility. The adjustment is made only for the kidney cancer component of total cancer risk because that is the tumor type for which the weight of evidence was sufficient to conclude that TCE-induced carcinogenesis operates through a mutagenic mode of action. However, it may be that TCE operates through a mutagenic mode of action for other cancer types as well or that it operates through other modes of action that might also convey increased early-life susceptibility. Additionally, the ADAFs from the 2005 Supplemental Guidance are not specific to TCE, and it is uncertain to what extent they reflect increased early-life susceptibility to kidney cancer from exposure to TCE, if increased early-life susceptibility occurs.

6.3. OVERALL CHARACTERIZATION OF TCE HAZARD AND DOSE RESPONSE

There is substantial potential for human exposure to TCE, as it has a widespread presence in ambient air, indoor air, soil, and groundwater. At the same time, humans are likely to be exposed to a variety of compounds that are either metabolites of TCE or have common metabolites or targets of toxicity. Once exposed, humans, as well as laboratory animal species, rapidly absorb TCE, which is then distributed to tissues via systemic circulation, extensively metabolized, and then excreted primarily in breath as unchanged TCE or CO_2, or in urine as metabolites.

Based on the available human epidemiologic data and experimental and mechanistic studies, it is concluded that TCE poses a potential human health hazard for noncancer toxicity to the CNS, the kidney, the liver, the immune system, the male reproductive system, and the developing fetus. The evidence is more limited for TCE toxicity to the respiratory tract and female reproductive system. Following EPA (2005b) *Guidelines for Carcinogen Risk Assessment*, 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 strong for NHL, but less convincing than for kidney cancer, and more limited for liver and biliary tract cancer. Less human evidence is found for an association between TCE exposure and other types of cancer, including bladder, esophageal, prostate, cervical, breast, and childhood leukemia. Further support for the characterization of TCE as "carcinogenic to humans" by all routes of exposure is derived from positive results in multiple rodent cancer bioassays in rats and mice of both sexes, similar toxicokinetics between rodents and humans, mechanistic data supporting a mutagenic mode of action for kidney tumors, and the lack of mechanistic data supporting the conclusion that any of the mode(s) of action for TCE-induced rodent tumors are irrelevant to humans.

As TCE toxicity and carcinogenicity are generally associated with TCE metabolism, susceptibility to TCE health effects may be modulated by factors affecting toxicokinetics, including lifestage, gender, genetic polymorphisms, race/ethnicity, preexisting health status, lifestyle, and nutrition status. In addition, while some of these factors are known risk factors for effects associated with TCE exposure, it is not known how TCE interacts with known risk factors for human diseases.

For noncancer effects, the most sensitive types of effects, based either on HECs/HEDs or on candidate RfCs/RfDs, appear to be developmental, kidney, and immunological (adult and developmental) effects. The neurological and reproductive effects appear to be about an order of magnitude less sensitive, with liver effects another 2 orders of magnitude less sensitive. The RfC of **0.0004 ppm** (0.4 ppb or 2 $\mu g/m^3$) is based on route-to-route extrapolated results from oral studies for the critical effects of heart malformations (rats) and immunotoxicity (mice). This RfC value is further supported by route-to-route extrapolated results from an oral study of toxic nephropathy (rats). Similarly, the RfD for noncancer effects of **0.0005 mg/kg/day** is based on the critical effects of heart malformations (rats), adult immunological effects (mice), and developmental immunotoxicity (mice), all from oral studies. This RfD value is further supported by results from an oral study for the effect of toxic nephropathy (rats) and route-to-route extrapolated results from an inhalation study for the effect of increased kidney weight (rats). There is high confidence in these noncancer reference values, as they are supported by moderate-to-high confidence estimates for multiple effects from multiple studies.

For cancer, the inhalation unit risk is **2×10^{-2} per ppm [4×10^{-6} per $\mu g/m^3$]**, based on human kidney cancer risks reported by Charbotel et al. (2006) and adjusted, using human epidemiologic data, for potential risk for NHL and liver cancer. The oral slope factor for cancer is **5×10^{-2} per mg/kg/day**, resulting from PBPK model-based route-to-route extrapolation of the inhalation unit risk estimate based on the human kidney cancer risks reported in Charbotel et al. (2006) and adjusted, using human epidemiologic data, for potential risk for NHL and liver cancer. There is high confidence in these unit risks for cancer, as they are based on good-quality human data, as well as being similar to unit risk estimates based on multiple rodent bioassays. There is both sufficient weight of evidence to conclude that TCE operates through a mutagenic mode of action for kidney tumors and a lack of TCE-specific quantitative data on early-life susceptibility. Generally, the application of ADAFs is recommended when assessing cancer risks for a carcinogen with a mutagenic mode of action. However, because the ADAF adjustment applies only to the kidney cancer component of the total risk estimate, it is likely to have a minimal impact on the total cancer risk except when exposures are primarily during early life.