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Trichloroethylene Health Risk Assessment: Synthesis and Characterization

NOTICE

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National Center for Environmental Assessment–Washington Office
Office of Research and Development
U.S. Environmental Protection Agency
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This document is a draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

This assessment presents EPA's most current evaluation of the potential health risks from exposure to TCE (trichloroethylene). TCE exposure is associated with several adverse health effects, including neurotoxicity, immunotoxicity, developmental toxicity, liver toxicity, kidney toxicity, endocrine effects, and several forms of cancer. Mechanistic research indicates that TCE-induced carcinogenesis is complex, involving multiple carcinogenic metabolites acting through multiple modes of action. Under EPA's proposed (1996) cancer guidelines, TCE can be characterized as "highly likely to produce cancer in humans."

For effects other than cancer, an oral RfD of 3×10^{-4} mg/kg-d was based on critical effects in the liver, kidney, and developing fetus. An inhalation RfC of 4×10^{-2} mg/m^3 was based on critical effects in the central nervous system, liver, and endocrine system. Several cancer slope factors were developed, with most between 2×10^{-2} and 4×10^{-1} per mg/kg-d. Several sources of uncertainty have been identified and quantified.

The mechanistic information suggests some risk factors that may make some populations more sensitive. There are suggestions that TCE could affect children and adults differently. In addition, several chemicals have the potential to alter TCE's metabolism and clearance and subsequent toxicity; conversely, TCE exposure can augment the toxicity of other chemicals. Widespread environmental exposure to some of TCE's metabolites makes it important to consider the cumulative effect of TCE along with other environmental contaminants.

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LIST OF ABBREVIATIONS

ADH	alcohol dehydrogenase
AIRS	Aerometric Information Retrieval System
ALDH	aldehyde dehydrogenase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
Cal/EPA	California Environmental Protection Agency
CEP	Cumulative Exposure Project
CH	chloral hydrate
CI	confidence interval
CYP2E1	cytochrome P450 2E1
CYP450	cytochrome P450
DCA	dichloroacetic acid, dichloroacetate
DCVC	dichlorovinyl cysteine
DCVG	dichlorovinyl glutathione
DEHP	diethylhexyl phthalate
DHEA	dehydroepiandrosterone
EPA	United States Environmental Protection Agency
GST	glutathione S-transferase
HPT	hypothalo-pituitary-testis (axis)
LOAEL	lowest-observed-adverse-effect level
mg/kg	milligrams per kilogram body weight
mg/kg-d	milligrams per kilogram body weight per day
mg/L	milligrams per liter
mg/m^3	milligrams per cubic meter
mM	millimolar (concentration)
mmol	millimoles
mRNA	messenger RNA
N	number (of cases)
NAT	N-acetyl transferase
NCI	National Cancer Institute
NHANES III	Third National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program

LIST OF ABBREVIATIONS (continued)

OR	odds ratio
PDH	pyruvate dehydrogenase
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
RfC	reference concentration
RfD	reference dose
RR	relative risk
RXR	retinoid-X receptor
SHBG	sex hormone binding globulin
TCA	trichloroacetic acid, trichloroacetate
TCE	trichloroethylene
TCOH	trichloroethanol
TEAM	Total Exposure Assessment Methodology
TLV	threshold limit value
TRI	Toxics Release Inventory
ug/L	micrograms per liter
ug/m^3	micrograms per cubic meter
VHL	von Hippel-Lindau tumor suppressor gene

PREFACE

This assessment presents EPA's most current evaluation of the potential health risks from exposure to trichloroethylene (TCE). TCE is a chemical solvent used for vapor degreasing of metals and as an intermediate in the manufacture of other chemicals. One of the chemicals most released into the environment, TCE is highly volatile, with most TCE released into the environment finding its way into the air. An important exception occurs when TCE is released into groundwater, where TCE can persist for years because of the limited contact between groundwater and air. When people are exposed to TCE, it is readily absorbed by all exposure routes and is widely distributed throughout the body.

The potential health risks of TCE in the environment have caused it to be listed as a chemical of concern across several environmental programs. TCE is listed as a hazardous air pollutant under the Clean Air Act, a toxic pollutant under the Clean Water Act, a contaminant under the Safe Drinking Water Act, a hazardous waste under the Resource Conservation and Recovery Act, and a hazardous substance under the Comprehensive Environmental Response, Compensation, and Liability Act (Superfund). It is a toxic chemical with reporting requirements under the Emergency Planning and Community Right-to-Know Act, and under the Toxic Substances Control Act certain releases must be reported to the Toxics Release Inventory.

This assessment draws on 16 state-of-the-science papers published as a supplemental issue of *Environmental Health Perspectives* (Wartenberg et al., 2000; Lash et al., 2000a; Pastino et al., 2000; Moore and Harrington-Brock, 2000; Bull, 2000; Green, 2000; Fisher, 2000; Bois, 2000a,b; Clewell et al., 2000; Boyes et al., 2000; Barton and Clewell, 2000; Chen, 2000; Rhomberg, 2000; Wu and Schaum, 2000), plus some other key references. Accordingly, this assessment focuses on analysis and interpretation rather than a compilation of study results. More detailed information on the epidemiologic and experimental studies of TCE can be found in the state-of-the-science papers and in comprehensive reviews compiled by ATSDR (1997) and EPA (1985, 1987).

As this assessment is written, it is being shaped by several new developments in risk assessment. The practice of risk assessment is evolving from a focus on one toxic effect of one pollutant in one environmental medium toward integrated assessments covering multiple effects and multiple media that incorporate information about mode of action, uncertainty, human variation, and cumulative effects of multiple pollutants in different media. This evolution responds to recommendations of the National Research Council (NRC, 1994), which have been embraced in EPA's proposed cancer guidelines (U.S. EPA, 1996).

Mode-of-action information is used throughout this assessment, both to understand the cancer hazard and to suggest common mechanisms with some of the effects other than cancer.

Mode-of-action information is also used to assess the relevance of the animal results and to identify risk factors for sensitive human populations.

Uncertainty analyses are used to quantitatively characterize the range of plausible risk estimates and to guide the choices made during the assessment. Uncertainty analyses can be particularly useful for explicitly characterizing the ability of the data and the models to provide stable, credible risk estimates.

Humans vary in their response to environmental contaminants, and risk assessors have been charged with using mode-of-action information to identify susceptible populations. Of special concern has been the potential for disproportionate risks to children. Executive Order 13045 (1997) requires "each Federal Agency shall make it a high priority to identify and assess environmental health and safety risks that may disproportionately affect children, and shall ensure that their policies, programs, and standards address disproportionate risks that result from environmental health risks or safety risks."

Human response to an environmental contaminant can be affected by other exposures and conditions that occur in a population. EPA (1997) guidance directs "each office to take into account cumulative risk issues in scoping and planning major risk assessments and to consider a broader scope that integrates multiple sources, effects, pathways, stressors, and populations for cumulative risk analyses in all cases for which relevant data are available."

This assessment evaluates the potential health risks of TCE in light of the available information and analyses pertinent to mode of action, uncertainty, human variation, and cumulative risks.

AUTHORS AND CONTRIBUTORS

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The final draft of this assessment was developed and written by **V. James Cogliano**, **Cheryl Siegel Scott**, and **Jane C. Caldwell** of EPA's National Center for Environmental Assessment (NCEA), a part of the Office of Research and Development. This work, however, would not have been possible without the significant contributions of many others, for which the authors are most appreciative.

Contributors

Quantitative analyses were contributed by **Chao W. Chen** (NCEA), **Jerry Blancato** (EPA's National Exposure Research Laboratory), and **Gina M. Pastino** (formerly with NCEA as a fellow of the American Association for the Advancement of Science). Earlier drafts of this assessment included contributions by **Jean C. Parker** (NCEA), **Robert P. Beliles** (NCEA), and **Kim-Chi Hoang** (formerly with NCEA, now with EPA Region 9 in San Francisco).

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This assessment has benefited from extensive peer involvement. Its conclusions draw from 16 state-of-the-science papers written by experts who have conducted research related to the assessment of TCE's health risks. These papers have been published as a supplemental issue of *Environmental Health Perspectives* (volume 108, supplement 2, May 2000). **Cheryl Siegel Scott** served as editor of this special issue and managed the overall development of the state-of-the-science papers. Funding was provided by EPA, the U.S. Air Force, the Occupational Safety and Health Administration, and private industry. The lead authors, affiliations listed in the state-of-the-science papers, and topics of the state-of-the-science papers include:

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Chao W. Chen, U.S. Environmental Protection Agency, Mechanism-based modeling for liver tumorigenesis.

Lorenz R. Rhomberg, Harvard School of Public Health, Dose-response analyses for liver, lung, and kidney tumorigenesis.

Chieh Wu, U.S. Environmental Protection Agency, Exposure assessment.

These authors have individually provided many constructive comments and suggestions for this synthesis and characterization, but were not asked for consensus on its overall conclusions.

Peer involvement was also facilitated by an External Involvement Group, composed of nine representatives from private industrial organizations, public interest groups, academic research institutions, and State and Federal agencies. These representatives were asked to (1) propose additional topics for state-of-the-science papers and secure expert scientists as authors, (2) review these additional papers for balance and completeness, (3) propose topics for synthesis and characterization, (4) suggest peer reviewers, and (5) keep scientists from their sector informed. The External Involvement Group includes:

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These representatives have also provided many constructive comments and suggestions for this synthesis and characterization, but were not asked for consensus on its overall conclusions.

Elizabeth Maull, **Paul Cammer**, and **Paul Dugard** were instrumental in identifying authors and providing funding for some state-of-the-science papers.

1. SUMMARY AND CONCLUSIONS

1.1. OVERVIEW OF TCE HEALTH HAZARDS

An extensive database—comprising epidemiologic studies, animal bioassays, metabolism studies, and mechanistic studies—shows that trichloroethylene (TCE) exposure is associated with a wide array of adverse health effects (see Section 3.4). TCE has the potential to induce neurotoxicity, immunotoxicity, developmental toxicity, liver toxicity, kidney toxicity, endocrine effects, and several forms of cancer. Overall, similar effects are seen by oral and inhalation exposures, suggesting that TCE or its active metabolites reach their target sites after absorption from either exposure route. Special attention has been given to investigating TCE's potential to cause cancer. Epidemiologic studies, considered as a whole, have associated TCE exposure with excess risks of kidney cancer, liver cancer, lympho-hematopoietic cancer, cervical cancer, and prostate cancer. TCE has been extensively tested in animals, with mice developing liver tumors, lung tumors, and lymphomas, and rats developing kidney tumors and testicular tumors. The epidemiologic evidence is strongest at sites where the animals develop cancer, with site concordance for kidney cancer (in rats and humans), liver cancer (in mice and humans), and lympho-hematopoietic cancer (in mice and humans). TCE is also associated with cervical cancer and prostate cancer in humans, sites for which there are no corresponding animal models.

Extensive metabolic studies show that exposure to TCE results in internal exposure to a complex mixture of TCE's metabolites (see Section 3.2). Much of TCE-induced toxicity may be attributable to its metabolites, as toxicity tests show that some metabolites cause effects similar to those of TCE and, in some assays, TCE is inactive in the absence of its metabolites. Depending on the effect, the pattern of TCE-induced toxicity does not match any single metabolite, but reflects a mixture. The metabolic studies also show that the pattern of metabolite formation is different at high and low doses of TCE, and that metabolism can be altered by levels of metabolites already in the body.

This last point takes on added significance in view of widespread environmental exposure to some of TCE's metabolites (see Section 2). These exposures can be either direct (for example, TCE's metabolites trichloroacetic acid [TCA] and dichloroacetic acid [DCA] are also major byproducts of drinking water disinfection) or indirect (for example, the dry cleaning solvent perchloroethylene can be metabolized to TCA). These common exposures give rise to a background level of some metabolites through which TCE may act. Accordingly, these other exposures should not be regarded as confounding exposures in the traditional sense. Rather, they may help explain why TCE is associated with toxic effects in one study but not in another. Determining background exposure to TCE and its metabolites is important to understanding TCE-induced toxicity (see Section 1.8).

Many of TCE's toxic metabolites are formed through the enzyme system that also metabolizes ethanol, acetaminophen, and many other drugs and environmental pollutants (see Section 3.2). Exposure to these chemicals (for example, recent alcohol consumption) can affect enzyme levels, temporarily altering or enhancing TCE's metabolism and toxicity in a manner analogous to drug interactions known for many pharmaceuticals (see Sections 1.6 and 3.3). In addition, enzyme activity can vary significantly between individuals, through both intrinsic factors (for example, genetics) and acquired factors (for example, disease) (see Sections 1.6 and 3.3). Thus, the effects of TCE can vary, depending on exposures and conditions specific to each person.

1.2. MODE OF ACTION

Mechanistic research on a few TCE metabolites has yielded insights into TCE's possible modes of action.[1] The research to date indicates that TCE-induced carcinogenesis is complex, involving multiple carcinogenic metabolites acting through multiple modes of action. Past explanations, such as the hypothesis linking mouse liver tumors to peroxisome proliferation, are not consistent with the whole of the data, and more complex hypotheses have been formulated (see Section 3.5).

TCE-induced mouse liver tumors may arise through multiple metabolites and multiple modes of action (see Section 3.5.1). The preponderance of evidence suggests that formation of TCE's CYP450 metabolites[2] is sufficient to explain the liver tumors caused by TCE (Bull, 2000; Chen, 2000). Exposure to the CYP450 metabolites chloral hydrate (CH), TCA, or DCA can cause liver tumors in mice, and DCA can also cause liver tumors in rats. A plausible mode of action is that TCE induces liver tumors through TCA and DCA modifying cell signaling systems

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[1]EPA's proposed cancer guidelines define *mode of action* as "a series of key events and processes starting with interaction of an agent with a cell, and proceeding through operational and anatomical changes resulting in cancer formation." A *key event* is "an empirically observed precursor step consistent with a mode of action." "Mode of action" is contrasted with "mechanism of action," which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. An agent may work by more than one mode of action, both at different sites and at the same tumor site (U.S. EPA, 1996, 1999). Mode-of-action data can be used to (1) assess the relevance of laboratory animal results to human environmental exposures, (2) identify risk factors and sensitive populations, (3) provide insight into the shape of the dose-response curve, and (4) quantify the relative sensitivity of laboratory animals and human populations.

[2]*Cytochrome P450* (CYP450) is a major system of enzymes that metabolize chemicals through oxidation. TCE metabolism is most strongly dependent on activity of one particular enzyme, CYP2E1. CYP2E1 activity has been found to vary among humans through both genetic and acquired factors, leading to differences in metabolism and sensitivity to TCE-induced toxicity. Involvement of CYP2E1 in TCE metabolism also raises the possibility that TCE metabolism and toxicity can be affected by exposure to other chemicals, as CYP2E1 activity can be induced by its role in metabolizing many drugs and environmental pollutants, including ethanol, acetaminophen, benzene, 1,3-butadiene, styrene, methylene chloride, chloroform, carbon tetrachloride, vinyl chloride, vinylidene chloride, and perchloroethylene (Pastino et al., 2000).

that control rates of cell division and cell death (Bull, 2000). Tumors from the TCA and DCA bioassays apparently arise from different mechanisms that can be distinguished from each other (see Section 3.5.1). Characterizing the tumors from the TCE bioassays as either TCA-mediated or DCA-mediated is a critical research need that limits understanding of the respective roles and interaction of TCA and DCA in the TCE-induced tumors (see Section 5).

There has been much less investigation of the mode of action of TCE-induced mouse lung tumors. It has been suggested that the lung tumors arise from rapid CYP2E1 metabolism of TCE in the Clara cells of the mouse lung that leads to an accumulation of CH and causes cell damage and compensatory cell replication that in turn leads to tumor formation (Green, 2000). Although this hypothesis is consistent with the higher level of CYP2E1 in mouse lung, it has not yet been determined whether accumulation of CH is a key event causing tumors or a coincidental event unrelated to the tumors (see Section 3.5.3). Moreover, several questions about this hypothesis remain unresolved. In addition, CH is clearly clastogenic and mutagenic at high doses (Moore and Harrington-Brock, 2000), raising the possibility of multiple modes of action.

TCE-induced rat kidney tumors may arise through multiple modes of action (see Section 3.5.2). The preponderance of evidence suggests that TCE's glutathione-S-transferase (GST) metabolites[3] are responsible for kidney toxicity and kidney tumors (Lash et al., 2000b). Although these metabolites have not been tested in cancer bioassays, the GST metabolite dichlorovinyl cysteine (DCVC) is mutagenic (Moore and Harrington-Brock, 2000). Cytotoxicity may also be involved, as cytotoxicity is observed in both rats and humans exposed to high levels of TCE (Lash et al., 2000b). Information is lacking on whether the CYP450 metabolites also have a role in kidney tumor development.

Although these possible modes of action are consistent with much of the mechanistic research that has been conducted, the explanations so far fall short of identifying the active metabolites and sequence of key events involved at each cancer site (see Section 3.5). Ambiguity about active metabolites and key events leads to uncertainty in extrapolating the proposed modes of action across species. For some suggested modes of action, it is possible that most humans may be less sensitive than rats or mice, and this differential sensitivity has been addressed in the dose-response assessment (see Sections 4.5.2, 4.5.3). Species-dependent differences in the kinetics of TCE's metabolism can bring about a different proportion of metabolites that depends on dose. This, in turn, can lead to different apparent modes of action across species and across dose ranges. For example, rat kidney tumors at high experimental doses may involve high-dose cytotoxicity, while rat and human kidney tumors at lower doses may arise through a mutagenic,

[3]*Glutathione–S–transferase* (*GST*) is an enzyme system that produces a minor quantity of TCE's metabolites. The potentially higher toxicity of these metabolites, however, may make them important agents of TCE's toxicity.

not cytotoxic, mode of action. Accordingly, it may not be appropriate to assume concordance of tumor site or mode of action across species or across dose ranges.

Recent mechanistic research has begun to investigate a more fundamental level of cellular activity (see Section 3.5), for example, TCE's effects on cell signaling and carbohydrate metabolism (Bull, 2000). Effects at this level could indirectly accelerate ongoing tumor development specific to each species. In this way, TCE could be an "opportunistic carcinogen" that can contribute to ongoing processes or conditions that induce different forms of cancer in different species or populations. This would imply that TCE poses a cancer risk to humans, although mechanism-specific animal models might not predict human cancer risks with much confidence.

1.3. WEIGHT OF EVIDENCE UNDER THE PROPOSED AND CURRENT CANCER GUIDELINES

Under EPA's proposed (1996, 1999) cancer guidelines, TCE can be characterized as "highly likely to produce cancer in humans" (see Section 3.6.2). Support for this characterization is strong, including (1) association of TCE exposure with increased risks of human kidney cancer, liver cancer, lympho-hematopoietic cancer, cervical cancer, and prostate cancer; (2) induction of multiple von Hippel-Lindau (VHL) gene mutations in renal cell carcinoma patients who had been exposed to TCE; (3) induction of kidney tumors and testicular tumors in rats and liver tumors, lung tumors, and lymphomas in mice; and (4) mechanistic information suggesting that the animal tumors arise through processes that may be relevant to humans. Under EPA's current (1986) cancer guidelines, TCE would be classified as a "probable human carcinogen" (group B1), with "limited" human evidence and "sufficient" animal evidence of carcinogenicity.

Two areas of much past discussion are the weight of the human evidence and the relevance of the animal tumors. Compared with previous TCE assessments, the weight of the epidemiologic evidence has become stronger with the state-of-the-science analysis by Wartenberg et al. (2000) (see Sections 3.4, 3.6.1). The epidemiologic studies have recently been augmented by molecular information, in which multiple mutations of the VHL tumor suppressor gene,[4] primarily C-to-T changes including nucleotide 454, were found in renal cell carcinoma patients with high, prolonged TCE exposure (Brüning et al., 1997b) (see Section 3.5.2). The

[4]The von Hippel-Lindau gene normally suppresses renal cell carcinomas. Mutations to this gene have been noted in kidney cancers and may be an important risk factor and mode of action for chemically induced renal cell cancer (Lash et al., 2000b). To illustrate the difference between "mode" of action and "mechanism" of action, knowledge that VHL gene mutations are involved may be enough to identify a plausible "mode" of action, whereas knowing how such modifications induce subsequent events leading to kidney cancer would be needed to identify the "mechanism" of action.

mechanistic research into the mode of action for each animal tumor site has begun to link TCE with disturbances in cell signaling and carbohydrate metabolism, which can lead to human cancer and other diseases (see Section 3.5). Subject to dose-response adjustments for relative human-to-animal sensitivity (see Sections 4.5.2, 4.5.3), this research makes it plausible that TCE acts through mechanisms that can cause cancer in humans.

These characterizations are consistent with recent assessments by other authoritative health agencies, including IARC, which classifies TCE as a "probable human carcinogen" (IARC 1995) and the National Toxicology Program (NTP), which classifies TCE as "reasonably anticipated to be a human carcinogen" (NTP, 2000a).[5] The NTP recently considered upgrading TCE to "known to be a human carcinogen," making a case for a stronger classification in the future (NTP, 2000b). Key research to support this classification includes further investigation of VHL gene mutations in other cohorts exposed to TCE (see Section 5). On the other hand, a sizable faction of scientists is not convinced of the relevance of the animal tumors or the strength of the epidemiologic studies, which only recently have accumulated enough power to detect associations between moderate occupational or residential TCE exposures and some relatively common cancers. After considering this alternative view, these health agencies have now converged in their assessment that TCE is "highly likely," "probable," or "reasonably anticipated to be" a human carcinogen.

1.4. QUANTITATIVE HEALTH REFERENCE VALUES

For effects other than cancer, an oral reference dose (RfD)[6] of 3×10^{-4} mg/kg-d was based on critical effects in the liver, kidney, and developing fetus (see Section 4.3.1). These effects were observed in subchronic studies in mice and rats at doses as low as 1 mg/kg-d (see Section 4.3.2). Because laboratory conditions do not necessarily represent lifetime human environmental exposure, including sensitive populations, this dose was adjusted from subchronic to lifetime exposure, from animals to humans, and from a general to a sensitive population (see Section 4.3.3). In addition, humans start higher on the dose-response curve than do animals, as humans are exposed to TCE's active metabolites from sources other than TCE, and this cumulative

[5]IARC's classification of TCE as a "probable human carcinogen" (their group 2A) is based on "limited evidence" of carcinogenicity in human studies and "sufficient evidence" in animal studies (IARC, 1995). The NTP's listing of TCE as "reasonably anticipated to be a human carcinogen" is based on "limited evidence of carcinogenicity from studies in humans, sufficient evidence of malignant tumor formation in experimental animals, and convincing relevant information that trichloroethylene acts through mechanisms indicating it would likely cause cancer in humans" (NTP, 2000a).

[6]A *reference dose* (*RfD*) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime (Barnes and Dourson, 1988).

exposure can alter TCE's metabolism and toxicity (see Sections 1.8, 3.3, 4.3.3). Differences between average and sensitive humans were estimated in an uncertainty analysis to be about 50-fold, and a further 100-fold overall adjustment was considered appropriate for the other differences (see Section 4.3.3). This latter 100-fold uncertainty indicates the potential for future research to reduce uncertainty and improve this assessment's accuracy (see Section 5). In the end, however, the RfD was calculated by reducing the 1 mg/kg-d dose by a factor of 3,000, representing the largest divisor used by EPA in the presence of substantial uncertainty (see Section 4.3.4).

An inhalation reference concentration (RfC)[7] of 4×10^{-2} mg/m^3 was based on critical effects in the central nervous system, liver, and endocrine system (see Section 4.4.1). Occupational studies showed adverse central nervous system effects in workers at a subchronic exposure to 7 ppm (38 mg/m^3) (see Section 4.4.2). To convert this to an RfC for lifetime exposure, this concentration was divided by a composite uncertainty factor of 1,000, representing a default of 10 for human variation and 100 for uncertainty in extrapolating from subchronic to lifetime exposure and effect levels to NOAELs (see Section 4.4.3).

The 3,000-fold and 1,000-fold uncertainty factors used in deriving the RfD and RfC, respectively, are not large, considering that the Agency for Toxic Substances and Disease Registry (ATSDR) (1997) has characterized the chronic studies as inadequate for supporting chronic health reference values.[8] The key uncertainty about the RfD and RfC is the relative lack of chronic studies, necessitating reliance on subchronic studies and the numerous attendant uncertainties in using subchronic studies to estimate health reference values for lifetime exposure. This assessment's use of pharmacokinetic modeling and route extrapolation allows comparison of a wider range of oral and inhalation studies, revealing that critical effects for both

[7]A *reference concentration* (*RfC*) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime (U.S. EPA, 1994).

[8]ATSDR (1997) concluded that the available chronic-duration studies had inadequate characterization of exposure, inadequate quantification of results, and lack of endpoints suitable for deriving chronic levels. ATSDR did calculate an intermediate-duration inhalation level of 0.1 ppm, based on neurologic effects (decreased wakefulness and decreased slow-wave sleep) in rats observed at a LOAEL of 50 ppm for 6 weeks (Arito, 1994). The corresponding human-equivalent concentration (44 ppm) was divided by a composite uncertainty factor of 300 (10 for human variability, 3 for extrapolation from animals to humans, and 10 for use of a LOAEL). ATSDR also calculated an acute-duration inhalation level of 2 ppm, based on neurologic effects (fatigue and drowsiness) in six human volunteers observed at a LOAEL of 200 ppm for 5 days (Stewart, 1970). The corresponding daylong concentration (58 ppm) was divided by a composite uncertainty factor of 30 (10 for human variability and 3 for use of a minimal LOAEL). ATSDR calculated an acute-duration oral level of 0.2 mg/kg-d, based on developmental effects (decreased rearing) in mice observed at a LOAEL of 50 mg/kg-d (Fredriksson, 1993). This was divided by a composite uncertainty factor of 300 (3 for human variability in TCE metabolism, 10 for extrapolation from animals to humans, and 10 for use of a LOAEL).

exposure routes fall within a narrow dose range and providing strength to both the RfD and RfC determinations.

Several cancer slope factors[9] were developed, with most slope factors falling between 2×10^{-2} and 4×10^{-1} per mg/kg-d. This range is supported by estimates derived for kidney cancer and liver cancer from occupational exposure to TCE, non-Hodgkin's lymphoma from exposure to drinking water contaminated with an average of 23 µg/L TCE, and liver cancer in laboratory mice exposed to TCE (see Sections 4.5.1, 4.5.2). Because this range is supported by diverse studies and does not include the highest or lowest estimates that were calculated, it can be considered rather robust and independent of the results or methods used to analyze any single study (see Section 4.5.6).

The range of cancer slope factors has not been reduced to a single number. A range is reasonable in view of the risk factors that can modify the effects of TCE in different populations (see Sections 1.6, 3.3). (Ranges were not developed for the RfD and RfC, because they include sensitive individuals by applying a factor to cover human variation.) For most cancer risk factors, however, data that would allow differential risks to be quantified are lacking. Only for the GST polymorphism that modifies the kidney cancer risk are data available, indicating a fourfold increased risk for the more sensitive population. Because the modifying effect of most risk factors cannot be quantified at this time, this assessment proposes instead that risk assessors use the upper end of the slope factor range for susceptible populations having risk factors for TCE-induced cancer. Although the extremes of the slope factor range are not based on data from more- or less-susceptible populations, this approach emphasizes the possibility of different risks in different circumstances, identifies risk factors that may increase susceptibility to TCE's effects, and provides a practical way to adjust risk estimates to reflect differential susceptibility.

The purpose of a risk assessment can also affect the choice of a value from the slope factor range. An assessment of maximum individual risk would use the upper end of the slope factor range, while an assessment of the number of cancer cases in a general population could use the midpoint of the range. This implies that the high end of the slope factor range is appropriate

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[9]A *slope factor* is an estimate of a carcinogen's potency, characterized as a plausible upper bound on the increased human cancer risk from lifetime exposure to an average dose of 1 mg/kg-d. That is, the slope factor estimates a bound on the risk per mg/kg-d, accordingly, the slope factor is expressed in units of inverse lifetime-average dose, or $(\text{mg/kg-d})^{-1}$. Multiplying a slope factor by a lifetime-average dose (in mg/kg-d) yields a plausible upper bound on the increased probability of developing cancer from exposure to the carcinogen. A *unit risk* is analogous to a slope factor, but expressed in units of inverse lifetime-average ambient air concentration $(\mu\text{g/m}^3)^{-1}$ or inverse lifetime-average drinking water concentration $(\mu\text{g/L})^{-1}$ instead of inverse lifetime-average dose $(\text{mg/kg-d})^{-1}$. Unit risks are convenient when exposure is expressed in terms of environmental concentrations (: g/m^3 or : g/L). Unit risk estimates are based on particular exposure assumptions, specifically, a 70-kg adult drinking 2 L/d and breathing 20 m^3/d. When applied to other populations with different exposure factors—for example, children—unit risk estimates should be adjusted accordingly.

for susceptible individuals but is less descriptive of the rest of the population. The 20-fold span of the slope factor range is smaller than the uncertainty in dose estimates from the pharmacokinetic models (see Table 4-1), suggesting that the range may, in some cases, underestimate high-end risk.

For less-than-lifetime exposure, current risk assessment practice typically assumes that cancer risk is proportional to exposure duration, for example, exposure for 7 years of a 70-year human lifespan would carry one-tenth the risk of lifetime exposure.[10] There is some evidence for TCE to suggest that this practice may underestimate the risk from less-than-lifetime exposures (see Section 4.5.2). Accordingly, tempering the assumption of duration-proportionality may be warranted in applications involving less-than-lifetime exposure.

The continuing development of revised cancer guidelines (U.S. EPA, 1996, 1999) has raised expectations that nonlinear approaches[11] may replace the linear extrapolation used to estimate cancer slope factors for TCE. This assessment has pursued nonlinear approaches for several tumor sites. The key limitation to the nonlinear analyses is the uncertain identity of the active metabolites and key events involved in TCE-induced cancer. This assessment's consideration of potential nonlinear approaches indicates that concern for cancer would extend down to doses of 3×10^{-4} and 8×10^{-4} mg/kg-d for liver tumors and testicular tumors, respectively (see Sections 4.5.2, 4.5.5). These doses are comparable to the RfD for effects other than cancer, indicating the importance of considering all health effects in any risk assessment of TCE.

1.5. UNCERTAINTY

Several sources of uncertainty have been identified and quantified for TCE.[12] Consideration of these uncertainties has shaped the choices made in this assessment and

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[10]Cancer risk is calculated by multiplying the slope factor by the lifetime average daily dose (*LADD*), defined as $LADD = (C \times IR \times ED) / (BW \times LT)$, where *C* is the concentration, *IR* the intake rate, *ED* the exposure duration, *BW* the body weight, and *LT* the lifetime (U.S. EPA, 1992a).

[11]Because nonlinear dose-response curves can have substantial *model uncertainty* below the experimental doses, these curves are not extrapolated below the observed data. Instead, the risk assessment discusses current understanding of the phenomena that may be occurring at lower doses. Factors to be considered include (1) nature of the observed response, (2) shape of the observed dose-response curve, (3) human sensitivity compared with experimental animals, (4) human variation in sensitivity, and (5) human exposure (U.S. EPA, 1996, 1999).

[12]Uncertainty arises because study conditions differ from conditions of human environmental exposure. Consequently, risk assessments typically involve several *extrapolations*: from laboratory animals to humans, from experimental doses to environmental levels, from one exposure route to another, from one exposure pattern to another, and from small samples to large, more heterogeneous populations.

One type of uncertainty stems from different interpretations of the available data (known as model uncertainty), another from estimation errors due to incomplete or imprecise data (parameter uncertainty).

An understanding of uncertainty can be used (1) to make more informed choices during the conduct of a risk assessment, and (2) to characterize the range of plausible risk estimates for different populations.

described the range of estimates that are consistent with existing data and current scientific understanding.

1.5.1. Model Uncertainty[13]

In this assessment, the two major areas of model uncertainty are pharmacokinetics and low-dose extrapolation.

The two pharmacokinetic models (Fisher, 2000; Clewell et al., 2000) reveal the existence of substantial model uncertainty, with risk estimates that differed by 15-fold (see Table 4-4). Fitting these models to additional data sets (Bois, 2000a, 2000b) produced calibrated models that yielded more compatible results and reduced this source of model uncertainty (see Section 4.2.1).

The choice between linear and nonlinear extrapolation[14] to low doses creates two distinct classes of estimates (see Sections 4.5.2, 4.5.3). This dichotomous uncertainty stems from different strongly held and widely held interpretations of the information on TCE's mode of action (see Section 3.5). Accordingly, this assessment used both linear and nonlinear approaches, the former to bound the risks and the latter to demonstrate the extent of uncertainty and value of further research into mode of action and human variation.[15] In addition, this assessment made efforts to develop risk estimates from human studies as an alternative to extrapolating from animal models. Average exposure in one human study was close to the occupational standard, reducing the uncertainty of high- to low-dose extrapolation.

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[13]*Model uncertainty* refers to a lack of knowledge needed to determine which scientific theory on which a model is based is correct. In risk assessment, model uncertainty is reflected in alternative choices for model structure, dose metrics, and extrapolation approaches. Other sources of model uncertainty concern whether surrogate data are appropriate, e.g., using data on adults to make inferences about children. The full extent of model uncertainty cannot be quantified, only models that have been analyzed. Model uncertainty is expressed through separate analyses yielding alternative estimates, not as a single estimate or distribution made by combining estimates from incompatible models. Combining results of incompatible models to create hybrid risk estimates can undermine the value of having preferred models (NRC, 1994, p. 174).

[14]*Linear extrapolation* implies that risk decreases proportionally with dose below the experimental data. *Sublinear extrapolation* (often called by the less specific term *nonlinear extrapolation*) implies that risk decreases more than proportionally.

Toxicologists often speak of a *threshold dose* below which an individual does not respond. Risk assessments generally do not use this term, as experimental data are not able to estimate thresholds with much confidence. For example, a response that is not statistically significant does not indicate a threshold, rather it can be consistent with a small risk that falls below the experiment's power of detection.

[15]Mode of action is not the only determinant of the shape of a dose-response curve. *Human variation* also affects the shape by spreading the responses in a heterogeneous population over a wider dose range (see footnotes 17, 21). Another factor is *background exposure*, which determines where to place an incremental environmental exposure on the overall dose-response curve (see footnote 26).

1.5.2. Parameter Uncertainty[16]

Uncertainty analyses and confidence intervals were developed for some of this assessment's key pharmacokinetic and dose-response parameters. Each description of parameter uncertainty assumes that the underlying model is valid.

Uncertainty in the pharmacokinetic data was analyzed and used to gauge which applications could be supported by stable models. Confidence intervals spanned approximately 100-, 5,000-, and 14,000-fold for doses in the liver, kidney, and lung, respectively (see Table 4-1, Section 4.2.2). The unstable model estimates for kidney and lung resulted in use of default scaling methods for these sites. For all sites, median dose estimates were used in subsequent calculations.

Uncertainty in the animal dose-response data is reflected by the ratio of ED_{10}s to LED_{10}s. These generally do not exceed a factor of 2 (Rhomberg, 2000).

1.5.3. Consideration of Multiple Areas of Uncertainty

How to combine these individual aspects of uncertainty is an unresolved question in risk assessment. Multiplying the sizes of the individual confidence intervals is not recommended, as this would yield an overall confidence interval whose extremes are too improbable and whose range is too wide to be of practical use. There are more defensible statistical approaches for combining multiple uncertain quantities, but these depend on understanding the interrelationships between the uncertain quantities and on addressing model uncertainty by assigning numerical weights to the different models. Because this question is unresolved, this assessment has adopted EPA's 3,000-fold limit on uncertainty factors for RfDs and RfCs and has developed a cancer slope factor range that reflects a middle range of estimates that are supported by several sources of data and several lines of reasoning. This implies, however, that the range may, in some cases, underestimate high-end risk.

It has been suggested that to facilitate probabilistic risk assessment, slope factors be replaced by slope factor distributions. The quantifiable uncertainties (for example, the twofold LED_{10}-to-ED_{10} range) are less important than others that have not been quantified (for example, pharmacokinetic model uncertainty and parameter uncertainties). To avoid misleadingly narrow uncertainty distributions that ignore the most important sources of uncertainty, this assessment does not recommend distributions based on uncertainties of secondary importance.

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[16]*Parameter uncertainty* refers to lack of knowledge about the values of a model's parameters. This leads to a distribution of values for each parameter. Common sources of parameter uncertainty include random measurement errors, systematic measurement errors, use of surrogate data instead of direct measurements, misclassification of exposure status, random sampling errors, and use of an unrepresentative sample. Most types of parameter uncertainty can be quantified by statistical analysis.

Consideration of nonlinear dose-response models draws attention to the uncertainty in estimating the point where the dose-response curve begins to turn sharply upward. Overestimating the dose where the curve turns sharply upward could lead to the error of declaring a dose "safe" when the risk actually could be quite high. Another consequence of using nonlinear dose-response curves is the need to determine background exposure. In the case of TCE, there is substantial background exposure to some of its toxic metabolites. If their dose-response curves are steep (as for DCA-induced liver tumors in mice), the incremental risk can be greater in a population with high background exposure (see Section 1.8).

The uncertainties quantified for TCE may be similar to what would be found for other chemicals if pertinent analyses were performed. Thus, the practical importance of describing TCE uncertainty is not to decrease confidence in the TCE estimates, but to understand that all risk estimates are uncertain, and that the true risks can be either higher or lower. This assessment uses central estimates, not extreme values, for several major sources of uncertainty. The resulting risk estimates are supported by several sources of data and several lines of reasoning, providing some measure of confidence that the risk estimates are robust and not likely to be substantially changed by a single new study or analysis.

1.6. HUMAN VARIATION AND SENSITIVE POPULATIONS

The mechanistic information on TCE indicates a potential for considerable human variation[17] and suggests some risk factors[18] that would make a population more sensitive. Further research is needed to estimate the magnitude of the increased risk (see Section 5).

1.6.1. Metabolic Differences

Because TCE's metabolites contribute to its toxicity, differences in metabolism can lead to differences in response (see Sections 3.2, 3.3). TCE's metabolism is extremely complex, and variation in one metabolic reaction can shift activity from one metabolic pathway to another,

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[17]*Human variation* refers to person-to-person differences in *susceptibility* due to differences in exposure or differences in biological *sensitivity*. Differences in biological sensitivity can be linked to inherited factors (e.g., genetics) or acquired factors (e.g., disease). Although human variation and uncertainty both can be characterized as ranges or distributions, they are fundamentally different concepts. Uncertainty can be reduced by further research that supports a model or improves a parameter estimate, but human variation is a fact that can be better characterized, but not reduced, by further research. Fields other than risk assessment use "variation" or "variability" to mean dispersion about a central value, including measurement errors and other random errors that risk assessors address as uncertainty.

[18]A *risk factor* is a condition (e.g., genetics or disease) or exposure (e.g., alcohol or chlorinated solvents) associated with a particular risk. For example, in the context of traffic accidents, known risk factors include alcohol consumption, excessive speed, and bad weather. In a particular case, causation is often attributed to a combination of risk factors.

yielding different proportions of metabolites that, in turn, yield different toxicity. Because of this complexity, metabolic differences may not lead to proportionate differences in risk, but do indicate a potential for differential risks.

For GST metabolism, genetic polymorphisms[19] have been identified and linked to an increased risk of TCE-induced kidney cancer (see Sections 3.3, 3.4.3). The California Environmental Protection Agency (Cal/EPA) (1999) has estimated that these may lead to fourfold differences in kidney cancer risk (see Section 4.5.1). For CYP2E1 metabolism, several investigators have found up to 50-fold variations in small groups of humans (see Section 3.3). This suggests a potential for considerable differences in response.

It has been asserted that increases in metabolic rate due to enzyme induction have little effect on the amount of metabolite formed in the liver (Kedderis, 1997).[20] This assertion was derived from physiologically based pharmacokinetic modeling exercises reported in a poster abstract (Kedderis, 1996). A consequence of this assertion is that first-pass metabolism from oral dosing would be virtually 100%, leaving no TCE to circulate throughout the body. More complex multiple-pathway pharmacokinetic models (Fisher, 2000; Clewell et al., 2000) show that this is not the case with concentrations of TCE circulating throughout the body being stored as fatty tissue. Moreover, even if the total amount of metabolites formed is unchanged, the balance between the rates of formation of GST metabolites and CYP450 metabolites would clearly be altered by a relative induction of one enzyme as opposed to another. These considerations suggest that enzyme induction and inhibition may be involved in susceptibility to the effects of TCE.

1.6.2. Disease

Some of TCE's effects may result from its ability to disturb carbohydrate metabolism and cell signaling (Bull, 2000). Diabetes is a disease caused by such disturbances, and diabetics are at greater risk for liver cancer, kidney cancer, and many other effects. Further, uncontrolled diabetes causes induction of CYP2E1, favoring increased formation of toxic metabolites. This suggests that diabetics may be sensitive to effects from TCE (see Section 3.3).

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[19]A *polymorphism* is the presence of two or more distinct types due to genetic variation in a population. A familiar example is the polymorphism of blood types O, A, B, and AB. A polymorphism of metabolic enzymes indicates variation in metabolic activity across groups of people. The genetic basis of polymorphisms implies that they can be differently distributed across racial or ethnic groups.

[20]Under this hypothesis, known as *blood flow limitation*, the metabolic rate without enzyme induction is so rapid that all TCE would be metabolized, hence metabolite production would be limited only by the amount of TCE present in the blood flowing into the liver. Increasing the metabolic rate would have no effect, as there would be no TCE left to metabolize.

Alcohol, like TCE, is metabolized by CYP2E1, alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (ALDH). Concurrent exposure to TCE and alcohol can exacerbate the metabolism and acute effects of both in a manner similar to acute drug-alcohol interactions known for many pharmaceuticals. More importantly, chronic alcohol consumption can induce long-term 20-fold increases in CYP2E1 levels, favoring increased formation of toxic metabolites (see Section 3.3).

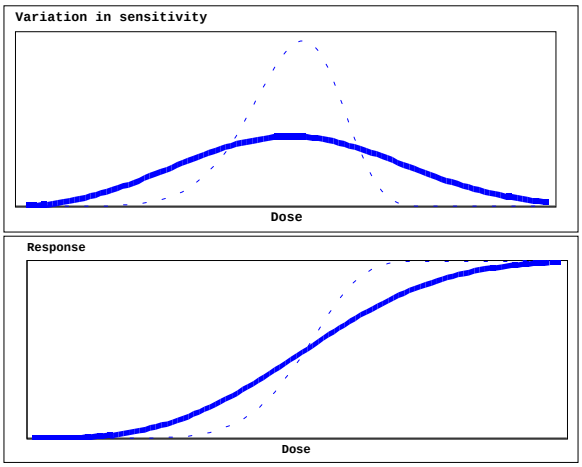
1.6.3. Gender Differences

TCE exposure has been linked to autoimmune diseases that occur mostly in women. In addition, recent epidemiologic studies have linked TCE exposure to cervical cancer. These differences in risk may be related to either pharmacokinetic or pharmacodynamic differences (see Section 3.3).

1.6.4. Some Implications

Identifying risk factors that vary across the human population allows us to begin identifying who is likely to be more sensitive. Human variation is a determinant of the shape of a population dose-response curve, which can be different from the dose-response curve for an individual.[21] Understanding the functional relationship between a risk factor and the associated disease is a critical research need that presently prevents estimating the differential risk faced by sensitive populations (see Section 5).

[21]To illustrate, consider two sensitivity distributions, centered at the same dose, for a homogeneous population (see thin line in upper figure) and a heterogeneous population (see thick line in upper figure). The corresponding population dose-response curves have different shapes, with the curve for the heterogeneous population spread over a wider range (see lower figure). This occurs even when the mode of action suggests a threshold, because the threshold would vary across a heterogeneous population and the population dose-response curve would be indistinguishable from those associated with nonthreshold models. In the human population, genetic and lifestyle factors contribute to variation in sensitivity that spreads the dose-response curve over a wider range (Lutz, 1990).



1.7. DIFFERENTIAL RISKS TO CHILDREN

There are several reasons to suspect that TCE could affect children and adults differently.[22]

1.7.1. Exposure Differences

Nursing is an exposure pathway unique to children. From its lipophilic nature, TCE would be expected to be present in milk. Limited data indicate that TCE has been detected in each of eight samples of human milk from four U.S. urban areas, but levels were not reported (ATSDR, 1997). Quantification of levels of TCE and its metabolites in milk, in both highly exposed and relatively unexposed populations, is a critical research need that presently prevents comparing levels in milk with levels allowed in drinking water (see Section 5). The widely accepted benefits of nursing highlight the value of minimizing the exposure of nursing mothers to TCE.

The cancer unit risk estimates (see Section 4.5) assume that mg/kg-d is an appropriate measure of dose and incorporate exposure factors that are based on adults (specifically, 2 L/d drinking water intake, 20 m^3/d air intake, and 70 kg body weight). Relative to body weight, however, children drink more water, eat more food, and breathe more air than do adults (U.S. EPA, 1999). When assessing risks from less-than-lifetime exposure that occurs during childhood, good exposure assessment practice would replace these adult exposure factors with childhood exposure factors (U.S. EPA, 1999).[23]

1.7.2. Pharmacokinetic Differences

Children—especially infants—and adults can have different levels of metabolizing enzymes (see Sections 3.2, 3.3). CYP2E1 is present in children, although levels are lower than in adults. The same is true for some enzymes that clear TCE and its metabolites from the body. Such differences may be responsible for early-life persistence in the body of trichloroethanol (TCOH), the only TCE metabolite studied in infants, children, and adults. Half-life is 8 days in adults, 10 days in children, 28 days in full-term infants, and 40 days in preterm infants (Renwick, 1998). This trend runs counter to the general expectation that smaller organisms metabolize and

[22]Children are not only a subpopulation, rather childhood is a sequence of life stages that affect the whole population. EPA has long been concerned about environmental risks to children and has identified areas where risks can be different in children and adults (Farland, 1992: (1) different exposures, (2) different remaining life expectancies (early exposures leave more time for latent effects to develop), (3) different internal doses from the same external dose (pharmacokinetic differences), and (4) different responses to the same internal dose (pharmacodynamic differences).

[23]For example, to adjust the drinking water unit risk for a 9-kg infant who drinks 1 L/d (instead of a 70-kg adult who drinks 2 L/d), multiply the unit risk by (1 L/d / 2 L/d) / (9 kg / 70 kg) = 3.9.

clear chemicals more rapidly; instead, TCOH persists longer in children than in adults, longer in infants than in children, and longer in preterm than in full-term infants. These differences do not necessarily imply greater sensitivity, as we do not know which metabolites are responsible for each adverse effect, but it does indicate a need for caution, as limited evidence suggests that TCE metabolism and clearance are different in infants and children compared to adults.

1.7.3. Pharmacodynamic Differences

TCE's neurotoxic potential suggests an increased risk during childhood, a period of rapid brain development (see Sections 3.4.1, 3.3). TCE's potential for developmental and endocrine effects also supports a concern for childhood exposure (see Section 3.4.5). Cancer, too, is an effect where children may be at higher risk. An accepted concept in carcinogenesis is that young animals are usually more susceptible to the carcinogenic activity of a chemical than are adults (McConnell et al., 1992).[24] Because TCE's metabolites can cross the placenta, the period of concern would extend to prenatal exposure. This concern is supported by a larger risk of childhood leukemia observed after maternal exposure to TCE-contaminated drinking water during the prenatal period (MA-DOH, 1997).

1.7.4. Some Implications

The health reference values developed in this assessment (see Sections 4.3–4.5) do not address the exposure, pharmacokinetic, and pharmacodynamic considerations discussed here. For example, the uncertainty factor for human variation is based on data collected in healthy adults. Some assurance that this assessment does not understate risks to children comes from consideration of sensitive test systems such as mouse liver. This sensitivity, though, is not related to the reasons that children and adults may differ. Consequently, it may be appropriate to consider a further factor to address the potential for differential risks to children (see Section 5).

1.8. CUMULATIVE RISKS INVOLVING TCE

Several chemicals have the potential to alter TCE's metabolism and clearance and subsequent toxicity. Much TCE-induced toxicity is attributed to its metabolites, and risks from TCE exposure can depend on background exposure to its metabolites. Some metabolites are highly present in the environment (see Section 2), from both direct sources (for example,

[24]Although much has been made of the qualitative finding that perinatal exposure rarely induces tumors not found with adult exposures, quantitatively, perinatal exposure in conjunction with adult exposure usually increases the incidence and reduces the latent period of a given tumor. For example, in a survey of 22 chemicals tested for perinatal exposure (TCE and its metabolites have not been so tested), a higher incidence of neoplasms was found when animals were exposed during the last third of pregnancy (McConnell et al., 1992).

ingestion of TCA and DCA as byproducts of drinking water disinfection) and indirect sources (for example, metabolism of other chlorinated solvents to TCA). Hence, these exposures and conditions can modify the effects of TCE on a population.[25]

At the same time, TCE exposure can augment toxicity induced by other chemicals. Among these, support is strongest for TCE to exacerbate the effects of concurrent exposure to alcohol or chlorinated solvents by inducing shared metabolic enzymes (see Section 3.3).

[25]Known as *cumulative risk assessment*, this area of inquiry focuses on a population and considers all chemicals and stressors that can affect the population. A cumulative risk assessment goes beyond the effects of each chemical individually to consider (1) how exposure to other chemicals and stressors can alter the toxicity of a chemical, (2) how exposure to a chemical can alter the toxicity of other chemicals and stressors, and (3) how genetics, life stage, lifestyle, diseases, and other conditions in an individual—factors associated with sensitivity—can affect expression of a chemical's toxicity. In this way, cumulative risk assessments consider human-chemical interactions as well as chemical-chemical interactions.

When a nonlinear dose-response curve is assumed, high background exposure can imply greater susceptibility to incremental exposures.[26] Consequently, an estimate of background exposure is needed to determine where to place an incremental exposure to TCE on the dose-response curve, as the size of the increased risk is different in different regions of the dose-response curve. Finding a safe dose in an otherwise unexposed population does not mean that that dose is safe when background exposures are considered. This aspect of susceptibility is a new feature that has not been addressed in past risk assessments, but will become important in future assessments that consider nonlinear dose-response curves (see Section 5).

This assessment has begun to address cumulative risk in several ways:

- Developing a pharmacokinetic model (at EPA's National Exposure Research Laboratory) for concurrent exposure to TCE, TCA, and DCA. This will enable risk assessors at different geographic sites to tailor the model to the cumulative exposures specific to their sites (see Section 4.2.1).
- Identifying diseases and other risk factors that may make some individuals more sensitive to TCE's effects (see Sections 1.6, 3.3).
- Including a modifying factor in the RfD to reflect that background exposures to TCE and its metabolites can be higher in humans than in laboratory animals (see Sections 1.4, 4.3.3). An analogous modifying factor was not used in the RfC because the exposed workers on which the RfC was based, unlike the laboratory animals on which the RfD was based, are likely to have had background exposures comparable to those of the general human population.
- Developing a range of cancer slope factors to permit risk assessors to choose a slope factor based on a population's risk factors and background exposures (see Sections 1.4, 4.5.6).

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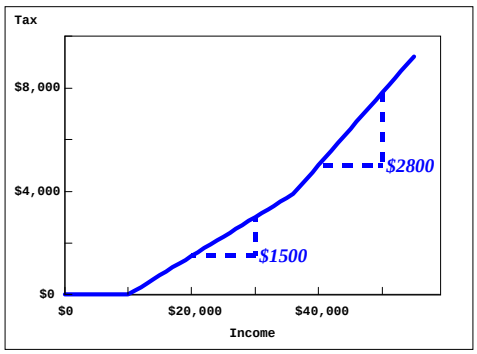
[26]The correlation between background exposure and susceptibility is a property of sublinear dose-response curves in general. A familiar analogy is how federal income tax rates increase with income. For example, the increased tax on $10,000 additional income can be (see figure at right):

$0 if there is no other income.
$1500 if added to $20,000 income.
$2800 if added to $40,000 income.

In this analogy, the same incremental income ("dose") results in a tax ("effect") that depends on background income.



2. EXPOSURE CHARACTERIZATION

This exposure characterization covers TCE, several metabolites of TCE, and other parent compounds that produce a similar profile of metabolites (see Figure 2-1). Direct exposure to TCE's metabolites or to parent compounds that produce these metabolites can alter or enhance TCE's metabolism and toxicity by generating higher internal metabolite concentrations than would result from TCE exposure by itself.

This characterization draws from the state-of-the-science paper on exposure (Wu and Schaum, 2000). More detailed exposure information can also be found in a supplemental report (Wu and Schaum, 2001).

2.1. ENVIRONMENTAL SOURCES

The major use of TCE is as a degreaser for metal cleaning operations. It is also used as a paint stripper, adhesive solvent, ingredient in paints and varnishes, and in the manufacture of organic chemicals. Releases from nonanthropogenic activities are negligible. TCE is on the list for reporting to the Toxics Release Inventory (TRI). Reported releases into air predominate over other types. Reported releases decreased by a third between 1987 and 1990 and have been stable since then.

TCE's metabolites and their other parent compounds are also generally used in a wide variety of manufacturing industries. Exceptions to this pattern are TCA and DCA, which are formed as byproducts of drinking water chlorination; 1,1-dichloroethane, which can be formed by biodegradation of 1,1,1-trichloroethane in landfills and other anaerobic environments; and perchloroethylene, whose releases have come primarily from the large number of local dry cleaning operations.

The TRI is the primary source of this information and can be considered recent and well documented. The major uncertainty is the number of minor releases that occur, which are exempt from reporting but, collectively, may be important.

2.2. ENVIRONMENTAL FATE

The chemical properties of TCE are well documented and widely accepted. Because of its high vapor pressure, TCE in the atmosphere is expected to be present primarily in the vapor phase rather than adsorbed to particles. Some removal from the atmosphere during wet precipitation is expected because of the moderate solubility of TCE in water (1.1 g/L). The major degradation process affecting vapor-phase TCE is photooxidation by hydroxyl radicals (half-life of 1–11 days).

The dominant fate of TCE released to surface soils or surface waters is volatilization to the air. Because of its moderate water solubility, TCE in soil (for example, landfills) has the potential to migrate into groundwater. The relatively frequent detection of TCE in groundwater confirms its mobility in soils. Biodegradation in soil and groundwater is thought to be slow (half-life on the order of months to years). For releases to surface waters, bioconcentration, biodegradation, and adsorption to sediments and suspended solids are thought to be insignificant.

2.3. EXPOSURE CONCENTRATIONS

No statistically based national sampling programs have been conducted that would allow estimates of true national means for any environmental medium. A substantial amount of air data, however, has been collected and summarized. TCE has been detected in the air throughout the United States. Ambient air measurement data can be obtained from the Aerometric Information Retrieval System (AIRS)[27], which contains about 1,200 measurements from 25 States during 1985–1998. Air levels in 1998 can be summarized as follows: range=0.01–3.9 $\mu g/m^3$, mean=0.88, median=0.32, 90th percentile=1.76.

TCE ambient air concentrations in 1990 were modeled for all census tracts of the continental United States as part of EPA's Cumulative Exposure Project (CEP)[28]. The modeling suggests that 97% of the census tracts have TCE concentrations ranging from 0 to 1.5 $\mu g/m^3$. The average level was estimated as 0.37 $\mu g/m^3$ and the maximum as 32 $\mu g/m^3$. The averages and percentiles can be interpreted as population-weighted values because all census tracts have roughly equal populations (but vary in geographic size). The modeling uses population weighting for its estimates of concentration within each census tract. CEP data suggest a pattern of generally low TCE levels in rural areas, higher levels in urban areas, and highest levels in small commercial or industrial sectors across most States. The pattern is consistent with the monitoring data.

These modeled values should be interpreted with caution. They are not as reliable as measured values for specific locations. AIRS data show an average for 1990 across 59 monitoring stations of 1.84 $\mu g/m^3$. This is much higher than the national average from CEP of 0.37 $\mu g/m^3$. An important difference, though, is that the CEP estimate represents all areas of the continental United States, whereas the 1990 AIRS data for TCE represent only 59 monitors located in eight States.

TCE has been reported in rainwater, surface water, groundwater, drinking water, and seawater. ATSDR reports that TCE is the most frequently reported organic contaminant in

[27]www.epa.gov/airsdata

[28]www.epa.gov/CumulativeExposure/air/air.htm

groundwater and has estimated that between 9% and 34% of drinking water supply sources have some TCE contamination. Most municipal water supplies that do not use contaminated groundwater, however, are in compliance with the maximum contaminant level of 5 µg/L.

TCE has been reported in marine sediments, marine invertebrates, marine mammals, foods, human milk, and human urine and blood. The food with the highest reported mean concentration is butter and margarine (73.6 ppb).

Biological monitoring is an important supplement to environmental monitoring for characterizing human exposure, as environmental monitoring data (especially for air) may not be representative of actual exposures. TCE has been most frequently detected in persons exposed through occupational degreasing operations. In 1982, TCE was detected in each of eight human milk samples from four U.S. urban areas. NHANES III reported that, from 1988 to 1994, TCE levels in whole blood were below the detection limit of 0.01 mg/L for about 90% of the people sampled.

2.4. EXPOSURE PATHWAYS AND LEVELS

Most people are exposed to TCE through drinking water, air, or food. TCE has been detected in human milk samples from the general population; consequently, nursing infants may be exposed. Also, because TCE can be present in soil, children may be exposed through activities such as playing in or ingesting soil.

AIRS monitoring data for 1998 indicate a mean outdoor air level of about 0.8 µg/m^3. Based on an inhalation rate of 20 m^3/d, intake by this pathway is about 18 µg/d. This is consistent with ATSDR, which reported an average daily air intake for the general population of 11–33 µg/d. Total inhalation exposures are likely to be higher, because limited studies suggest that indoor air may contribute more to overall exposure than outdoor air.

A survey of large water utilities in California for 1984 found a median concentration of 3 µg/L. Based on a water consumption rate of 2 L/d, intake by this pathway is about 6 µg/d. This is consistent with ATSDR, which reported an average daily water intake for the general population of 2–20 : g/d.

TCE can be found in groundwater. It is not known how often TCE reaches levels of concern, but the highest potential for this to happen would be at wells located near TCE disposal or contamination sites where leaching occurs. TCE is a common contaminant at Superfund sites. It has been identified in at least 852 of the 1,416 hazardous waste sites proposed for inclusion on the EPA National Priorities List. About 41 million people live less than 4 miles from a Superfund site.

TCE exposures may be elevated for people living near waste sites, residents of some urban or industrialized areas, people exposed at work, and people using products containing TCE

with poor ventilation. In addition, TCE is transferred from shower water to air (mean efficiency, 61%); ATSDR reported that a 10-minute shower in TCE-contaminated water could result in inhalation exposure comparable to that from drinking TCE-contaminated tap water.

Table 2-1 presents adult exposure estimates for TCE and several of its metabolites and other parent compounds that also produce those metabolites. Of special note is that general-population exposures to TCA and DCA are each approximately 10-fold higher than exposure to TCE. This indicates that there is a high background level of these metabolites that not only can alter and enhance the metabolism of TCE, but also have toxic effects themselves. This topic is further discussed in Section 3.

The general population exposure estimates are derived directly from environmental media data and, therefore, have the same uncertainties as described above. In addition, inhalation exposures are probably low, because they are based on ambient air measurements, which do not reflect indoor levels. It is likely that indoor levels are generally higher and contribute more to inhalation exposure than does outdoor air. For example, the 1987 EPA TEAM (Total Exposure Assessment Methodology) study shows that the ratio of indoor to outdoor TCE concentrations for Greensboro, NC, was about 5:1. The prevalence of TCE exposure on the basis of blood levels is based on NHANES III, which was recent (1988 to 1994) and fairly extensive (644 individuals).

Table 2-1. Preliminary dose estimates of TCE and TCE-related chemicals

Chemical	Population	Medium	Range of estimated adult exposures (μg/day)	Range of estimated adult doses (mg/kg/day)	Data sources
Trichloroethylene	General	Air	11 – 33	1.57E-04 – 4.71E-04	(*3*)
	General	Water	2 – 20	2.86E-05 – 2.86E-04	(*3*)
	Worker	Air	2,232 – 9,489	3.19E-02 – 1.36E-01	(*3*)
Tetrachloroethylene	General	Air	80 – 200	1.14E-03 – 2.86E-03	(*13*)
	General	Water	0.1 – 0.2	1.43E-06 – 2.86E-06	(*13*)
	Worker	Air	5,897 – 219,685	8.43E-02 – 3.14	(*13*)
1,1,1-Trichloroethane	General	Air	10.8 – 108	1.54E-04 – 1.54E-03	(*16*)
	General	Water	0.38 – 4.2	5.5E-06 – 6.00E-05	(*16*)
1,2-Dichloroethylene	General	Air	1 – 6	1.43E-05 – 8.57E-05	(*18*)
	General	Water	2.2	3.14E-05	(*18*)
cis-1,2-Dichloroethylene	General	Air	5.4	7.71E-05	(*6*)
	General	Water	0.5 – 5.4	7.14E-06 – 7.71E-05	(*6*)
1,1,1,2-Tetrachloroethane	General	Air	142	2.03E-03	(*6*)
1,1-Dichloroethane	General	Air	4	5.71E-05	(*7*)
	General	Water	2.47 – 469.38	3.53E-05 – 6.71E-03	(*7*)
Chloral	General	Water	0.02 – 36.4	2.86E-07 – 5.20E-04	(*6*)
Monochloroacetic acid	General	Water	2 – 2.4	2.86E-05 – 3.43E-05	(*19*)
Dichloroacetic acid	General	Water	10 – 266	1.43E-04 – 3.80E-03	(*19*)
Trichloroacetic acid	General	Water	8.56 – 322	1.22E-03 – 4.60E-03	(*19*)

3. HAZARD ASSESSMENT AND CHARACTERIZATION

3.1. GENERAL APPROACH

The database for TCE is relatively rich in toxicity information that includes epidemiologic studies, animal bioassays, metabolism studies, genetic toxicity studies, and mechanistic studies. Interpretation of this information, especially the cancer studies, has sparked controversies concerning the strength of the human evidence and the human relevance of the animal responses. These controversies have prompted some new epidemiologic studies and reviews, plus a wealth of mechanistic research committed to developing and supporting hypotheses about TCE's potential modes of action in animals.

To help provide a sound scientific basis for addressing these and other issues, this assessment commissioned a set of state-of-the-science papers that appears in a May 2000 supplement of *Environmental Health Perspectives*. One paper addresses the controversy over the weight of the human evidence. Wartenberg et al. (2000) conducted a joint analysis of the cancer epidemiology studies, using a statistically based weight-of-evidence approach that stratified the available studies into tiers according to how well each study's results can be associated with TCE exposure specifically. Such an analysis affords an opportunity to put differing results in perspective by evaluating whether the positive or the nonpositive results overall have greater weight, and whether the overall result is statistically significant.

Other state-of-the-science papers focus on describing the effects of TCE and its metabolites as well as the human relevance of observed animal responses. Lash et al. (2000a) describe the metabolism of TCE, providing a foundation for the consideration of various metabolites and target sites throughout the body. Pastino et al. (2000) discuss factors that can cause humans to vary in their susceptibility to adverse health effects from TCE exposure and identify populations that are likely to be more susceptible. Moore and Harrington-Brock (2000) discuss the genetic toxicity of TCE and its metabolites, a critical step in understanding TCE's mode of action at different sites. Bull (2000), Green (2000), and Lash et al. (2000b) discuss potential modes of action for tumorigenesis in the liver, lung, and kidney, respectively. These latter papers reflect the considerable research effort that has gone into developing the modes of action for cancer for their respective tumor sites, described as targets in past TCE assessments (U.S. EPA, 1985, 1987). The paper of Barton and Clewell (2000) reviews the evidence in the rodent for effects other than cancer (discussed in this section), and also provides support for the development of an inhalation reference concentration and oral reference dose (see Section 4).

Bull's state-of-the-science paper (Bull, 2000) has turned attention toward cell signaling to give insight into possible modes of action for TCE. This in turn, has helped provide focus for common modes and targets for cancer and other forms of toxicity. Many potential targets of

TCE toxicity (e.g., heart, brain, testes, and the developing fetus) may be affected by disturbances in carbohydrate metabolism and alterations in cell signaling, thus, common themes may ultimately emerge for different targets and effects. Particularly relevant is the work of Bannasch (Bannasch et al., 1984, 1986, 1997; Bannasch, 1986, 1996) demonstrating that disturbance of carbohydrate metabolism and cell signaling may be an early marker and common feature of carcinogenesis. Discussions with the state-of-the-science authors have also directed attention to the potential role in the toxicity of TCE of activation of the peroxisomal proliferator-activated receptor by TCE or its metabolites. They also suggested a focus on the rationale supporting linear or nonlinear carcinogenic responses in the liver. Accordingly, an expanded mode-of-action section includes a detailed discussion of potentially relevant epigenetic mechanisms of carcinogenesis.

This hazard assessment focuses on analysis and interpretation rather than a compilation of study results. More detailed information on the epidemiologic and experimental studies on TCE can be found in the state-of-the-science papers and in comprehensive reviews compiled by ATSDR (1997), IARC (1995), and EPA (1985, 1987). Considerable new literature has become available on TCE's potential toxicity and modes of action, including several effects other than cancer. Some of the new information postdates the publication of the state-of-the-science papers and is integrated into this hazard assessment. Thus, the discussion of toxicity and mode of action is considered more current and the recent references expand the scope beyond cancer. Section 3.2 examines TCE's metabolism and clearance, because TCE-induced toxicity is often attributed to TCE's metabolites. This leads to a discussion in Section 3.3 of susceptibility, because several features of TCE metabolism and clearance have been found to be differentially distributed across the general population, suggesting some potentially susceptible populations. Section 3.4 summarizes the toxic effects reported from TCE exposure. These effects are grouped by organ system, and for each organ system, cancer and other effects are discussed together. Each summary of TCE-induced effects is followed by a summary of available information about similar effects reported in studies of TCE's metabolites. Section 3.5 discusses some potential modes of action through which TCE may induce these effects. A mode of action can involve several systems, and conversely, effects in one system may arise from several distinct modes of action. Much of the research supporting the hypothesized modes of action has concentrated on a few TCE metabolites rather than on TCE itself.

3.2. METABOLISM

The state-of-the-science paper by Lash et al. (2000a) discusses the absorption, distribution, metabolism, and excretion of TCE and its metabolites. TCE is rapidly and extensively absorbed by all routes of environmental exposure—ingestion, inhalation, and skin

contact. Once absorbed, TCE is distributed via the circulatory system throughout the body, where it can accumulate in fat and other tissues. Storage of TCE in fat represents an internal source of exposure that can later release TCE again into the circulation. The pathways of biotransformation observed in humans and animals are thought to be similar qualitatively (Pastino et al., 2000).

TCE is metabolized primarily in the liver, but metabolism can also occur in other tissues. Metabolism in these other tissues may be important to TCE-induced toxicity. The kidney is the only other tissue that has been extensively studied to any extent. Metabolism occurs through two main pathways (oxidation or conjugation with glutathione) (see Figure 3-1). Although several metabolic products derived from these pathways have been identified for TCE, only a few have been characterized pharmacokinetically (Lash et al., 2000a). The first major pathway begins with oxidation of TCE by at least four isozymes of the cytochrome P450 (CYP450) system to CH, which is then acted upon by ADH and ALDH to form trichloroethanol (TCOH) and TCA, respectively. CYP450 is also most likely involved in the reduction of CH to TCOH with TCA as a product. TCOH undergoes glucuronidation and is either excreted in the urine or persists in the body through enterohepatic recirculation.[29] TCOH can then be excreted from the liver to the small intestines, where it is reabsorbed into the circulation. In general, enterohepatic recirculation is more extensive in humans than rodents (Barton and Clewell, 2000), with the result of a larger area-under-the-curve (AUC) in humans than rodents for an equivalent oral dose. Overall, the rate-limiting step for this pathway is oxidation of TCE to CH (Lash et al., 2000a).

DCA is another metabolite detected in mice and humans, but the source of DCA formation is not clear. DCA may result from the oxidative pathway by further oxidation of both TCA and TCOH or by rearrangement directly from dichloroacetyl chloride, from glutathione conjugation, or, likely, from both pathways (Lash et al., 2000a; Cai and Guengerich, 2000; Völkel et al., 1998). The estimation of the amount of DCA formed in humans contains uncertainty because there are problems associated with the analytical methodology, particularly in the presence of large amounts of TCA, and because of DCA's relatively rapid metabolism to oxalic acid, monochloroacetic acid, glycolic acid, and glyoxylic acid. Conversion to glyoxylic acid is thought to be by a newly described isoform of glutathione S-transferase (GST), identified as GST zeta (GSTZ) (Lash et al., 2000a). GSTZ enzyme activity in vitro differs between rats and humans and is relatively more sensitive to inhibition by DCA in rats (Cornett et al., 1999). In general, GSTs are present in multiple forms in mammals and have a large degree of overlapping

[29]*Enterohepatic recirculation* is the process by which metabolites formed from the biotransformation of the parent compound in the liver are excreted directly into the bile without first entering systemic circulation. From the bile, the metabolites pass into the small intestine and are available for reabsorption (Klaussen, 1980).

substrate specificity among the differing forms. They also exhibit polymorphisms and species differences in expression and catalytic activities. Hence, the further characterization of this pathway as it relates to TCE is important.

The second major pathway of TCE metabolism also involves GST and begins with glutathione conjugation, primarily in the liver, to S-(1,2-dichlorovinyl)glutathione (DCVG). DCVG formed in the liver is excreted in the bile and converted in the biliary tract and intestines to S-(1,2-dichlorovinyl)-L-cysteine (DCVC). DCVC is translocated to the kidneys, where its concentration modulates the amount of toxic metabolites generated in the kidney. DCVC can be detoxified by N-acetyltransferase (NAT) in the kidney and excreted in the urine, or it can be metabolically activated to a thioacetylating agent by $-lyase. Although the capacity of this pathway is small compared with the CYP450 pathway, the chemically unstable and highly reactive nature of the metabolites produced by $-lyase metabolism make it likely that relatively small amounts can induce significant responses (Lash et al., 2000a).

Quantitatively, the comparative rate of TCE metabolic reactions between species or between potential target organs is a complex question. The interspecies relationship of TCE metabolic pathways has been investigated using pharmacokinetic models (Clewell et al., 2000; Fisher, 2000) and analyses that look at uncertainty in those models (Bois, 2000a,b). Peak blood levels of TCE's oxidative metabolites (e.g., TCA) are higher in mice and rats than in humans administered equivalent doses, whereas blood levels of TCE in humans generally remain for a longer time period, i.e., longer half-life. Differences in enterohepatic recirculation and metabolism could be factors in interspecies differences. These factors result in greater persistence in the body and a larger AUC for some TCE metabolites in humans compared to rodents. For the GST conjugation pathways in the kidney, in vitro work suggests that DCVG formation in human liver and kidney was only slightly lower than in the rat, with DCVG formation in mice shown to be markedly faster than in either humans or rats (Lash et al., 2000a). However, DCVG formation is only the initial step in the generation of nephrotoxic species, with additional studies necessary to characterize this metabolic pathway (Lash et al., 2000a). A gender difference also is apparent, with an overall higher GSH conjugation in male rats than in female rats (Lash et al., 2000a).

3.3. SUSCEPTIBILITY

The state-of-the-science paper by Pastino et al. (2000) discusses physiologic factors (e.g., genetics, gender, and age) and acquired factors (e.g., disease, alcohol consumption, and exposure to other solvents) that may cause humans to vary in their susceptibility to adverse health effects from TCE exposure. Another way to view the factors that can influence susceptibility to potential TCE-related toxicity is to categorize them as either intrinsic or extrinsic. Inherent

differences in an individual's genetic makeup, gender, age, or disease state may affect susceptibility intrinsically, whereas coexposure to a pollutant that alters their metabolism or clearance, or that adds on to background levels of metabolites, may affect susceptibility extrinsically. Through either mechanism, increased susceptibility to potential TCE-related toxicity may occur by altering the body burden of TCE or its metabolites, sensitizing the individual to other agents that act in common metabolic pathways, or augmenting underlying conditions that share common effects with TCE (e.g., effects on cell signaling).

The variation observed for specific metabolic reactions or responses within individual populations suggests potential for considerable overall differences in response between individuals in the whole population. It is, however, difficult to describe quantitatively the overall extent of human variability, as the range of variation in a single process or metabolic reaction, as well as variation within a specific ethnic group, may not encompass the span of variation across the entire population or take into account the multiplicity of factors that can determine the toxicity of TCE. Nonetheless, the variation observed for specific metabolic reactions or groups not only suggests a potential for considerable overall differences in response between individuals, but can be used to identify populations and conditions that may be associated with a higher risk from TCE exposure.

Human variation is also a determinant of the shape of a population dose-response curve. The dose-response curve for a susceptible population can be different and changed when compared to the dose-response curve for the heterogeneous population. Additionally, direct or background exposure to some of TCE's metabolites can be considerable and, for individuals with these exposures, can aggravate the effect of small incremental exposures of TCE. That is, background and cumulative exposures place the susceptible population on a different part of the dose-response curve. For example, given a metabolite with an "S"-shaped dose-response curve, a small dose would induce a minimal response in the absence of background exposure, yet the same dose could induce a greater response in the presence of background exposure that places an individual higher on the "S"-shaped curve, in the range where the risk increases rapidly with dose.

3.3.1. Intrinsic Factors Affecting Susceptibility

3.3.1.1. *Differences in Metabolism and Clearance*

Adverse effects resulting from TCE exposure are believed to be attributed mainly to its metabolites. Hence, individual differences in metabolism can lead to individual differences in response. The influence of physiologic and acquired factors on metabolism, particularly on

CYP2E1,[30] which may be responsible for more than 60% of TCE metabolism, is important (Lipscomb et al., 1997). Rates for TCE metabolism by the oxidative pathway are believed to vary across the population. In vitro, an eightfold variation in metabolic activity of TCE via CYP2E1 was seen in 23 human liver samples (Lipscomb et al., 1997); several investigators have found activity of CYP2E1, in general, to vary up to 50-fold in humans (Stevens et al., 1994; Peters et al., 1990; Yoo et al., 1998; Raucy, 1995; Lieber 1997; Pastino et al., 2000).

For the GST pathway,[31] genetic polymorphisms may not only influence metabolism of TCE and its target of toxicity. One epidemiologic study has linked polymorphisms of the GST pathway (GSTT and GSTM) to increased risk of kidney cancer from TCE exposure (Brüning et al., 1997a). Additionally, one isozyme, GSTZ, is important to the further metabolism of DCA (Tong et al., 1998), and in vitro has been shown to be irreversibly inhibited in mice, rats, and humans with chronic DCA exposure (Tzeng et al., 2000). In vitro studies of cytosolic GST conjugation of TCE in humans have revealed considerable (3-8 fold) variation in relatively small samples (Lash et al., 2000a). In general, regulation of GST expression differs among tissues, such that not all GST isoforms are expressed in every tissue. Partial deletions are also found to varying degrees in specific ethnic populations (15%–20% of Caucasians and 60% of Asians having partial deletions). Therefore, it is difficult to accurately predict expression of GST genes in a given tissue (Eaton and Bammler, 1999).

3.3.1.2. *Disease States*

Certain diseases may increase susceptibility to TCE's adverse effects through induction of metabolic enzymes, favoring increased formation of toxic metabolites of TCE, or by adding to ongoing physiological processes. For example, uncontrolled diabetes causes induction of CYP2E1 (Wang, 2000; Nakajima, 2000). Furthermore, diabetics may be a population susceptible to TCE's adverse effects on the basis of recent mechanistic understanding about the

[30]CYP2E1 is one of the CYP450 enzymes and is the primary catalyst of hydrocarbon bioactivation in animals and, most likely, in humans as well (Raucy et al., 1993). CYP2E1 also metabolizes many drugs and environmental pollutants, including ethanol, acetaminophen, benzene, 1,3-butadiene, styrene, methylene chloride, chloroform, carbon tetrachloride, vinyl chloride, vinylidene chloride, and perchloroethylene. Exposure to these chemicals can affect enzyme levels or activity and, in so doing, alter or enhance TCE's toxicity. CYP2E1 is found in greatest concentration in liver, but is also expressed in parenchymal and non-parenchymal cells of the liver (e.g., Kupffer cells) and in many extrahepatic tissues (e.g., kidney, brain, testes, lung, and bone marrow).

[31]GST enzymes are involved in metabolizing many drugs and environmental pollutants, including polycyclic aromatic hydrocarbons, phenolic antioxidants, reactive oxygen species, isothiocyanate, trivalent arsenicals, barbiturates, and synthetic glucocorticoids (Hayes and Pulford, 1995). GST metabolism aids in the clearance of xenobiotics and is generally considered a detoxification process. This is not the case for trichloroethylene, where highly reactive metabolites can be produced.

relationship between TCE and its metabolites and carbohydrate handling and cell signaling (Bull, 2000) (see Section 3.5.1).

3.3.1.3. *Gender*

Gender-related differences in metabolism may influence susceptibility by shifting production of metabolites from one toxic pathway to another, or by providing differing targets for TCE's toxicity. Males have been reported to have higher rates of DCVG formation from acute exposure (Lash et al., 2000a). There may also be gender-related differences in target organs. One recent case-control study (Dosemeci et al., 1999) observed an elevated risk among females, but not males, between kidney cancer and occupational TCE exposure. This study was not able to address potential gender differences in level of exposure, however. Females have also shown a greater susceptibility than males to some of TCE's immune system effects (Sanders et al., 1992; Barton and Clewell, 2000). Moreover, gender-specific organs may be targets of TCE's effects. TCE exposure has been associated with excess risks of cervical cancer in occupationally or environmentally exposed women (Blair et al., 1998; Anttila et al., 1995; Burg, 1997), and in male rats, has been shown to concentrate in the testes (Zenick et al., 1984), decrease serum testosterone, and alter testicular steroid precursors (Kumar et al., 2000).

3.3.1.4. *Age*

Several inherent aspects of uptake, distribution, and clearance of TCE in children can predispose children to its toxicity. In general, children are believed to metabolize and clear xenobiotics faster than adults, although rate differences vary across chemicals and the patterns of metabolism may be different from those of adults. In contrast to this general expectation, the half-life of TCOH in the body has been reported to be 20% higher in children, 3.5-fold higher in full-term infants, and fivefold higher in preterm infants compared with healthy adults (Renwick, 1998). This finding, which could extend to other TCE metabolites, may be due to differing enzyme activities in children. For example, glucuronidation does not reach adult levels until 3 to 6 months of age. Individual children may also have low levels of a particular isoform, as shown by their inability to efficiently carry out glucuronidation and their increased risk for developing hyperbilirubenemia. Such children may also be less able to glucuronidate TCOH. Furthermore, ADH activity has been shown to be decreased in the neonate (Pastino et al., 2000). Other life stage differences, such as the amount of body fat in an organ, can also lead to differences in TCE distribution and accumulation. The brain of the newborn contains more lipid than that of adults, thus affecting the distribution of TCE.

The prenatal and postnatal periods of development may be critical for TCE's potential carcinogenic and noncarcinogenic toxicity. Although the modes of action of the carcinogenicity

of TCE are unknown, if TCE behaves similarly to the limited number of chemicals tested during perinatal exposure as well as adult exposure, the carcinogenic potential of TCE may also be greater in the young (McConnell, 1992). Moreover, TCE exposure to mice during the perinatal period has been reported to be associated with long-lasting alterations in spontaneous motor activity (Fredriksson et al., 1993), although this report is only suggestive because of its lack of statistical analysis on the litter. Exposure during the prenatal period has been shown to induce toxicity. For example, animal studies report associations between maternal exposure to TCE in drinking water and cardiac malformations in offspring (Johnson et al., 1998a,b; Dawson et al., 1993; Epstein et al., 1993). Finally, the longer life expectancy of children compared with adults may lead to a longer period for expression of toxicity.

3.3.2. Extrinsic Factors Affecting Susceptibility

3.3.2.1. *Alteration of Metabolism and Clearance Through Coexposure to Other Chemicals*

Exogenous exposures that induce CYP450 enzymes can enhance TCE metabolism and toxicity. Cytochrome P450, particularly CYP2E1, is important to the metabolism of many exogenous chemicals, including solvents and ethanol; CYP2E1 is also readily induced by a large number of these substrates. For example, ethanol is metabolized by CYP2E1, alcohol dehydrogenase, and aldehyde dehydrogenase. Although interactions have been demonstrated in the toxicity of both ethanol and TCE given simultaneously at relatively high levels for short periods, more profound effects on TCE toxicity are liable to occur from chronic ethanol exposure. Chronic ethanol consumption can result in 20-fold induction of CYP2E1 (Pastino et al., 2000; Fisher, 2000), significant because large numbers of people consume ethanol. In addition, ethanol is a weak inducer of peroxisomes, with ethanol consumption linked with increased risk of liver cancer (Falk, 1982; NIH, 1985). Thus, exposure to both ethanol and TCE may create conditions that increase risk through shared pathways of metabolism, induction of metabolic enzymes, and potential targets of toxicity. Methanol is often a concurrent exposure for TCE and shows affinity for many of the same enzymes. Furthermore, TCE exposure can increase the toxicity associated with methanol and ethanol exposure by altering not only metabolism to aldehydes but also their detoxification. For example, TCE exposure has been reported to induce an “alcohol-flush,” similar to those who possess an inactive ALDH allele (as seen in half of individuals of Asian heritage), which may be due to TCE’s ability to decrease ALDH activities at relatively low concentrations (Wang et al., 1999). In addition, the induction of P450 metabolism or aldehyde clearance by TCE may increase aldehyde production as well (Wang et al., 1999).

3.3.2.2. *Additive Effects from Coexposure to Other Chemicals*

The addition of TCE exposure to other background exposure has the potential to increase susceptibility to TCE's adverse effects. Exposure to perchloroethylene, the dry cleaning solvent, can be considerable in some populations, and TCA is a primary metabolite of perchloroethylene. Moreover, TCA and DCA may be found in drinking water, with background exposures to TCA and DCA each about 10-fold higher than background exposure to TCE (Wu and Schaum, 2000).

3.3.2.3. *Age*

Children and infants may be more susceptible to TCE's toxicity because they may receive higher exposures via the mother either through systemic circulation during pregnancy or mother's milk, as well as from pharmacokinetic differences between a developing child and adult. Limited data collected from residents in U.S. urban areas have indicated that TCE is present in human milk (ATSDR, 1997); nursing infants may receive a larger exposure to TCE owing to its presence in mother's milk (Fisher et al., 1989). The fetus may also serve as a reservoir for maternally derived metabolites; CYP2E1 has been reported to be expressed in full-term placentas (Vieira et al., 1998). TCA has been reported to accumulate in mouse fetuses and amniotic fluid (Danielsson, 1990; Ghantous et al., 1986). DCA also readily crosses the placenta to the fetus (Smith et al., 1992).

3.4. TOXICITY

TCE affects many organs and systems of the body, consistent with its lipophilic nature and ability to distribute widely throughout the body. This section summarizes effects reported for exposure to TCE and its metabolites, presented by organ system. A discussion of mechanisms and modes of action are discussed in Section 3.5 because they are, in several cases, applicable to more than one system.

3.4.1. Neurotoxicity

The ability of TCE to cause neurotoxic effects is well established. In general, TCE produces a "solvent narcosis" that may be related to effects on membrane fluidity and may include anesthetic effects. TCE was formerly used as a general anesthetic and induces this effect at about 2,000 ppm (ASTDR, 1997). The array of symptoms observed with TCE exposure is similar to other solvents, such as alcohol, ethers, petroleum distillates, and other halogenated solvents.

Acute or short-term inhalation of TCE has been associated with dizziness, headache, sleepiness, nausea, confusion, blurred vision, and weakness in several human studies cited by Barton and Das (1996). TCE is distributed to the brain, and brain concentrations of TCE in the rat tend to mimic blood concentrations (Boyes et al., 2000). Acute neurologic effects of TCE in

both humans and rats are more closely associated with peak concentrations rather than AUC (Boyes et al., 2000), but rats require a higher peak concentration to elicit these effects (Bushnell, 1997). Hence, humans appear to be a sensitive species for the neurotoxic effects of TCE.

Longer term or chronic inhalation exposure is associated in a number of human studies with a similar array of neurological symptoms as those observed with acute exposure (ATSDR, 1997). One study (Vandervort et al., 1973), used by Cal/EPA (1999) to derive a reference exposure level (similar to an RfC), reported eye irritation, drowsiness, heart palpitations, cough, weakness, and dizziness among workers exposed to a wide range of TCE concentrations. Other studies of occupational exposure to TCE have reported similar LOAELs (see Table 4-3). Tremors have been reported in several inhalation studies (Grandjean et al., 1955; Liu et al., 1988; Bardodej and Vyskocil, 1956) and in one study of a population exposed to a mixture of TCE and perchloroethylene in drinking water (White et al., 1997). Long-term inhalation exposure at an average concentration of 44 ppm TCE has also been associated with early nerve function impairment (Ruitjen et al., 1991).

It is not clear whether some of TCE's neurotoxic effects may be attributed to TCE as the parent or to one of its metabolites such as DCA, which has shown longer term effects on the central nervous system. A recent study (Moser et al., 1999) in two strains of rats exposed to DCA for up to 2 years showed neuromuscular toxicity and mild tremors with neurotoxicity being route, duration, and strain dependent. Drinking water exposure was shown to be more toxic than gavage, with effects seen at the lowest drinking water dose (16 mg/kg-d). In addition, neurotoxicity from DCA was described as more pronounced and persistent, and occurring at lower doses than previously reported (Moser et al., 1999). Subchronic exposures to DCA in drinking water are associated with brain lesions in dogs at doses of 12.5 mg/kg-d or higher (Cicmanec et al., 1991) and rats at doses of 125 mg/kg-d or higher (Bhat et al., 1991; Katz et al., 1981). At much higher doses, focal reactive gliosis—proliferation of reactive astrocytes after dead neurons have been cleared away—and vacuolization were observed in the brains of DCA-treated rats (Bhat et al., 1991). DCA given orally is also reported to decrease nerve conduction velocity in tibial nerves in terms of areas, perimeters, and diameter in rats (Yount et al., 1982) and, in a human case report, to induce a peripheral neuropathy (Moore et al., 1979).

3.4.2. Liver

3.4.2.1. *General Hepatotoxicity*

TCE can induce hepatotoxicity in humans, as reported in several studies cited by ATSDR (1997). This is not unexpected, as most metabolism of TCE occurs in the liver. TCE can affect liver functions not reflected in a standard battery of liver function tests. Workplace exposure to TCE has been reported to change serum cholesterol and increase bile acids (Nagaya et al., 1993;

Driscoll et al., 1992). Alterations of plasma bile acids have been noted in workers at inhalation exposures of less than 5 ppm (Driscoll et al., 1992; Neghab et al., 1997). Elevations of serum bile acids, possibly an early sign of liver dysfunction, have also been seen in exposed rats (Wang and Stacey, 1990; Hamdan and Stacey, 1993). Alterations of cholesterol metabolism have been observed in workers with low-level chronic TCE exposure, effects that persisted after 2 years (Nagaya et al., 1993). TCE exposure also can alter insulin and endocrine profiles in occupationally exposed individuals (mean, 30 ppm) (Chia et al., 1997; Goh et al., 1998), effects that may be important to responses of the liver.

Increased liver weight, primarily resulting from cytomegaly, is one of the most frequently reported effects in animals dosed chronically with TCE by either the inhalation or oral route. Barton and Clewell (2000) summarize several studies in which orally administered TCE changed the liver-weight-to-body-weight ratio in rats and mice. These effects have been observed at gavage doses as low as 50 mg/kg-d for 14 days (Berman et al., 1995). TCE was reported to increase cell replication but not cytotoxicity or reparative hyperplasia. TCE metabolites can also stimulate cell replication; TCA, DCA, and CH have all been associated with increased liver size (Bull, 2000).

Although the state-of-the-science papers have focused on the effects of TCE on parenchymal cells of the liver, nonparenchymal cells may also have a role in TCE hepatotoxicity. TCE and DCA have been reported to alter Kupffer cell morphology, indicating activation, in mice and dogs, respectively (Kjellstrand et al., 1983; Katz et al., 1981). Kupffer cells[32] contain CYP2E1, which is highly inducible, and have been shown to be critical in the toxicity of ethanol, acetaminophen, phenobarbital, galactosamine, and carbon tetrachloride (Laskin et al., 1988, 1986, 1995; Knecht et al., 1995; Laskin, 1996; Lieber, 1997).

3.4.2.2. *Liver Cancer*

The review of the TCE cancer epidemiology finds an overall excess incidence of liver cancer in the tier-I studies (RR=1.9, 95% CI=1.0–3.4, N=12) and the tier-II studies (RR=2.0, 95% CI=1.3–3.3, N=15), with null results in the tier-III studies (Wartenberg et al., 2000). Although none of the tier-I studies achieves statistical significance individually, the relative risks exceed 1.0 in all three incidence studies, and in the only study that reported mortality for this

[32]Kupffer cells, which remove particulate material from the blood and drain the digestive system before it enters systemic circulation, can release potent mediators such as eicosinoids, toxic cytokines, proteolytic enzymes, superoxide anion, and tumor necrosis factor and be involved in hepatotoxicity (Caldwell-Kenkel et al., 1991). Biologically active mediators released by activated macrophages have been implicated in tumor promotion, as well as regulation of CYP450-mediated drug biotransformation (Laskin et al., 1988). Kupffer cells may also have implications for increased toxicity or increased likelihood of tumor progression if their role in immune and endocrine function is altered.

specific site. There is a biological gradient seen in the Anttila study (Anttila et al., 1995), as liver cancer incidence increased with increasing exposure and with increasing time since first exposure. Among those with the longest time since first exposure, relative risks are large (RR=6.1, 95% CI=2.8–17.7). A more recent study (Ritz, 1999) supports the observations in the Anttila study. Although the study was based on few deaths, risks increased with increasing potential TCE exposure and with increasing time since first exposure. Case-control studies are supportive, generally, of statistically significant associations with organic solvents as a class.

These findings are supported by the well-established and widely acknowledged result that TCE causes liver tumors in male and female mice (see Table 3-1). The NTP conducted two gavage studies in B6C3F1 mice (NCI, 1976; NTP, 1990), and Maltoni conducted inhalation studies in Swiss mice and B6C3F1 mice (Maltoni et al., 1986, 1988). In each study, TCE exposure caused a statistically significant increased incidence of liver tumors in male and female mice. Neither series of studies, however, observed liver tumors in rats.

Previously, it had been suggested that the liver tumors might be caused by epichlorohydrin, 1,2-epoxybutane, or other stabilizers present with the TCE (Henschler et al., 1980). Subsequent testing, however, has ruled this out (Henschler et al., 1984). The state-of-the-science papers (Lash et al., 2000a; Bull, 2000) suggest that TCE induces liver tumors through its metabolites. TCA and DCA, as well as CH, have all been shown to cause liver tumors in mice (Bull, 2000), with DCA also causing liver tumors in rats (DeAngelo et al., 1996).

3.4.3. Kidney

3.4.3.1. *General Nephrotoxicity*

A range of kidney toxicity following human exposure to TCE has been reported in several studies cited by ATSDR (1997) and Lash et al. (2000b). Tubular damage, as assessed by altered excretion of urinary proteins, has been noted in occupationally exposed individuals (ATSDR, 1997), as well as in renal cell carcinoma patients with high-level occupational exposure to trichloroethylene (Brüning et al., 1999a,b). TCE causes dose-related nephrotoxicity in male and female rats and mice (NTP, 1988, 1990; Maltoni et al., 1986, 1988). The lesions are not the chronic interstitial nephrosis usually observed in aged rats, but consist of cytomegaly, kayomegaly, and toxic nephrosis of tubular epithelial cells in the inner renal cortex (Lash et al., 2000b). As in the liver, TCE can change the kidney-weight-to-body-weight ratio in rats, mice, and gerbils (Barton and Clewell, 2000; Maltoni et al., 1986, 1988; Kjellstrand et al., 1981, 1983).

3.4.3.2. *Kidney Cancer*

Consistency across epidemiological studies is strongest for an association between TCE exposure and kidney cancer (Wartenberg et al., 2000). There is an overall excess of kidney

cancer incidence in the tier-I studies (RR=1.7, 95% CI=1.1–2.7, N=21) and tier-II studies (RR=3.7, 95% CI=1.7-8.1, N=6). Mortality was also elevated in the tier-III studies (RR=2.3, 95% CI=1.5–3.50, N=20), but not incidence. The relative risk exceeds 1.0 in three of four studies in tier 1 that reported incidence, one of which was a follow-up cluster report and achieved statistical significance individually. It is not obvious that index cases were excluded from these studies. Supporting the tier-I findings are the tier-II studies and case-control studies of kidney cancer and TCE (Vamvakas et al., 1998; Dosemeci et al., 1999), degreasers (Schlehofer et al., 1995), and solvents (Mellemgaard et al., 1994). Dosemeci (Dosemeci et al., 1999) and Blair (Blair et al., 1998) observed differences in risk between men and women, with higher risk in women. Dosemeci (Dosemeci et al., 1999) puts forth several hypotheses, including a possible gender-related difference in the distribution of polymorphisms of the GST metabolic pathway or differences in level of exposure. The report of Brüning (Brüning et al., 1997a) showed an increased risk for kidney cancer among individuals with GST isozymes M1 and T1, suggesting the GST pathway and polymorphisms of this pathway as important contributors to kidney cancer risk (Lash et al., 2000b).

Recently, occupational exposure to TCE has been linked to somatic mutations of the VHL gene[33] (Brüning, 1997b; Brauch et al., 1999). Thirty-three of the 44 renal cell carcinomas had one or more mutations in the VHL gene, predominately characterized as changes in cytosine to thymidine (Brauch et al., 1999). An interesting observation was a mutation in cytosine at a specific point in the VHL gene, at nucleotide (nt) 454, in 13 of these tumors; none of the 107 renal cell carcinomas from individuals not exposed to TCE displayed this mutation. High levels of exposure were associated with a higher frequency of tumors with multiple mutations and with the mutation at nucleotide 454 than lower exposure. This mutation was, moreover, found in normal kidney tumor tissue that was adjacent to neoplastic tissue in four patients. This observation suggests that a mutation at nucleotide 454 may precede tumor development. Both the studies of Brüning (1997b) and Brauch et al. (1999) point to an association of increased occurrence of mutated sequence in the entire gene for TCE-exposed renal cancer patients, with the work of Brauch indicating multiple mutations and a specificity in where and how mutations occurred within the nucleotide sequence of this gene.

Another study (Schraml, 1999) assessed the entire genome of 12 renal cell tumors from individuals with suspected exposure to TCE and other organic solvents (exposure was not described in this report, nor was an exposure score assigned to individual cases) and observed

[33]The VHL gene, consisting of hundreds of nucleotides, normally suppresses renal cell carcinoma. Somatic alterations to the gene are noted in sporadic kidney cancers, and mutation of the VHL gene is considered a risk factor for kidney cancer (Lash et al., 2000b).

mutations to genes on several chromosomes besides chromosome 3, the location of the VHL gene. Mutations found on these other chromosomes included both DNA losses and DNA gains. There were no differences between TCE and non-TCE exposed renal cell tumors regarding which chromosomes were affected. Schraml (1999), however, observed a much lower frequency of mutation of the VHL gene (25%, 4 tumors) among the 12 tumors from TCE-exposed individuals compared to either the report of Brauch et al. (1999) or to background rates (around 60% of all renal cell carcinomas) (Brauch et al., 1999). This study provides only limited information because of its absence of complete information on exposure and on tumor stage or grade, its smaller sample size, and its lower than expected number of tumors with VHL mutations.

The human results are supported by the animal studies, where TCE causes kidney cancer in rats by both ingestion and inhalation exposure (see Table 3-2). Maltoni observed a significantly increased incidence of kidney adenocarcinomas in male Sprague-Dawley rats inhaling TCE (Maltoni et al., 1986, 1988). The NTP conducted parallel corn-oil gavage studies in ACI, August, Marshall, and Osborne-Mendel rats (NTP, 1988), and a similar study in F344/N rats (NTP, 1990). The NTP considered the former to be inadequate studies of carcinogenic activity because of chemically induced toxicity, reduced survival, and deficiencies in the conduct of the studies. Despite these limitations, however, the NTP concluded that there were tubular cell neoplasms of the kidney in rats exposed to TCE. Consistent with these results, an earlier NCI study also reported a tubular adenocarcinoma in one male Osborne-Mendel rat exposed to TCE for 78 weeks (NCI, 1976).

As noted by the NTP (1988), the survival of exposed rats was substantially reduced compared with controls. Consequently, the tumor incidences tabulated in the NTP report may be misleading, because animals dying early may not be at full risk of developing tumors. To ascertain the effect of this reduced survival on the tumor incidences, the individual animal tumor pathology in the NTP report was used to adjust the tumor incidences to reflect the number of animals at risk. Animals were discounted if they died before the first kidney tumor was observed, at 57 weeks. Counting only animals alive at 57 weeks leads to the corrected incidences reported in Table 3-2.

Rhomberg (2000) analyzed the findings of TCE bioassays in rats and suggests that females are as sensitive as males to TCE-induced renal carcinogenicity. Previously, the kidney tumors in male rats had received more attention than those in females (ATSDR, 1997). Consideration of all strains together in Table 3-2, however, reveals that there is virtually no difference between males and females in the overall incidence of these tumors.

3.4.4. Immune and Lympho-Hematopoietic Systems

3.4.4.1. *General Effects on the Immune and Lympho-Hematopoietic System*

Immune system[34] changes have been noted in individuals presumed to have been exposed to TCE and other chlorinated solvents in drinking water (ATSDR, 1997). The ability of TCE to alter immune responses has been observed in mice exposed to TCE via drinking water or inhalation (Khan et al., 1995; Aranyi et al., 1986; Hobara et al., 1984; Parks et al., 1993; Sanders et al., 1982; Tucker et al., 1982). Mice exposed to TCE in drinking water at 0.1 mg/mL and higher showed inhibition of humoral and cell-mediated immunity, as well as effects on macrophage function and monocyte-granulocyte progenitor cells (Sanders et al., 1982). Females showed a greater susceptibility than males to these immunotoxic effects of TCE (Sanders et al., 1982; Barton and Clewell, 2000).

TCE and one of its metabolites have also been shown to affect bone marrow function or components. Sanders et al. (1982) observed an inhibition of stem cell colonization in both male and female mice. Lock et al. (1996) reported that DCVC causes renal toxicity and fatal aplastic anemia in calves at a single dose of 4 mg/kg, a dose much larger than that expected from TCE metabolism, and that toxicity may be related to the $-lyase metabolic pathway (Anderson and Schultze, 1965; Bhattacharya and Schultze, 1967). However, aplastic anemia has not been reported from similar exposures in rat, guinea pig, dog, or cat (Lock et al., 1996).

TCE exposure may also accelerate an autoimmune response. Several studies of occupational exposure have associated TCE and organic solvent exposure with several autoimmune diseases:[35] systemic sclerosis (scleroderma), fascitis, and systemic lupus erythematosus (SLE) (Lockey et al., 1987; Saihian et al., 1978; Niertert et al., 1998; Bovenzi et al., 1995; Goldman, 1996; Schaeverbeke et al., 1995; Waller et al., 1994). Furthermore, two studies of populations exposed to TCE and other solvents in drinking water report changes in T-lymphocytes and increased autoantibodies (Byers et al., 1988; Kilburn and Warshaw, 1992). Autoimmune-disease-prone mice exposed to 2.5 or 5.0 mg/mL TCE in drinking water show an increase in autoimmune antinuclear antibodies, elevated serum levels of several immunoglobulins, activation of CD+4, and a cytokine profile consistent with a TH1 type

[34]Immune cells are derived from stem cells in the bone marrow or fetal liver and are widely distributed throughout the body (Selgrade, 1995). They are also controlled by cytokines. The immune system is highly conserved across species such that organs and cells of the immune system in humans, mice, and rats are similar. In instances where controlled human studies have been possible, results of immune function studies in mice have been accurate predictors of effects in humans.

[35]Autoimmunity can be defined as a loss of self-tolerance that results in an immune reaction against oneself or self-antigens. Autoimmunity appears to have a multifactorial etiology that includes genetic and environmental factors (chemicals and microbes) (D'Cruz, 2000).

response[36] (Gilbert, 1999; Griffin et al., 2000a,b). In another recent drinking water study (Griffin et al., 2000c), these investigators noted many of these same observations, but these effects occurred at much lower exposures to TCE than previously reported; doses in this latter study were 0.1 mg/mL, 0.5 mg/mL, and 2.5 mg/mL. An early response to TCE exposure was a dose-related and significant increase in serum antinuclear antibodies at the lowest exposure (0.1 mg/mL, or 4 mg/kg/d). Furthermore, histopathologic changes that included portal infiltration by mononuclear cells were seen at the termination of the study, 32 weeks, and were consistent with the induction of autoimmune disease in the liver (Griffin et al., 2000c). Additionally, hepatocyte reactive changes were observed at all exposures including the lowest tested dose (0.1 mg/mL, or 4 mg/kg/d).

3.4.4.2. *Lymphoid Cancer*

Overall, an increased incidence of lympho-hematopoietic cancer (RR=1.4, 95% CI=1.0–2.0, N=40) was noted in tier-I by Wartenberg et al. (2000), primarily a reflection of the elevated, but not statistically significant, incidence of non-Hodgkin's lymphoma (RR=1.5, 95% CI=0.9–2.3, N=22). Null results were seen in the tier-II and tier-III studies (Wartenberg et al., 2000). Two drinking water studies of ecologic design provide some evidence of an association between non-Hodgkin's lymphoma and drinking water that includes TCE and other chemically similar solvents (Fagliano et al., 1990; Vartianen et al., 1993). Both studies were of ecological design without individual exposure or residential histories. The case-control studies that examined occupational exposures were not informative, primarily because of few cases and, consequently, lower statistical power. However, case-control studies that evaluated lymphoid cancer (leukemia and non-Hodgkin's lymphoma) and exposure to drinking water contaminated with TCE report elevated risks, although not all risks were statistically significant (Cohn et al., 1994; MA-DOH, 1997). One study of childhood leukemia (MA-DOH, 1997) observed a very strong association with exposure during pregnancy (OR=13.2, 95% CI=0.9-205.2). For both leukemia and non-Hodgkin's lymphoma, these studies report exposure-response gradients supporting drinking water contamination as the etiologic agent (Cohn et al., 1994; MA-DOH, 1997). Exposures are not sufficiently specific to a single chemical, although TCE is often the chemical found in highest concentration and the others have similar metabolism and targets.

TCE induces malignant lymphomas in mice in a number of inhalation or gavage bioassays. Mice have been shown to be an excellent immunopathologic model for human

[36]The profile of cytokines secreted by activated CD4+ T cells is often measured as an indicator of the type of immune response induced by a toxicant. A T-helper type 1 (TH1) type response reflects macrophage activation and inflammation and is marked by an increased secretion of IFN-(with a simultaneous decrease in the secretion of the cytokine IL-4 (Griffin et al., 2000a).

lymphoid cancers (Pattengale, 1986, 1994). Henschler found a statistically significant increased incidence of malignant lymphomas in female Han:NMRI mice exposed by inhalation (Henschler et al., 1980). The NTP found a significant increase in malignant lymphomas in female B6C3F1 mice exposed by gavage ($p<0.05$ by life-table test), although this result was not mentioned in the abstract or summary of peer review comments (NTP, 1990). Consistent results were found in the NCI study (NCI, 1976). Lymphosarcomas and malignant lymphomas were just beginning to appear in exposed mice when the NCI study was terminated at 90 weeks, although the numbers were too few for statistical significance. For comparison, in the 104-week NTP study only 1 of the 13 malignant lymphomas in exposed female mice was observed before week 90, at week 85.

3.4.5. Developmental and Reproductive Toxicity, Endocrine Effects

3.4.5.1. *Developmental Effects*

Epidemiologic studies of women exposed to degreasing solvents, including TCE, have reported elevated risks for cardiac anomalies in their offspring (Wilson et al., 1998; Goldberg et al., 1990; Ferencz et al., 1997). Large, statistically significant excesses were observed for specific cardiac defects: left-sided obstructive defects (OR=6.0, 95% CI=1.7-21.3) and hypoplastic left heart (OR=3.4, 95% CI=1.6-6.9) with an attributable risk[37] of 4.6% (Wilson et al., 1998; Ferencz et al., 1997). Neural tube defects have also been noted with either occupational or drinking water exposure to solvents including TCE (Bove et al., 1992; Holmberg, 1980a,b, 1982).

TCE or its metabolites, TCA and DCA, cause cardiac malformations and eye malformations (anopthalmia, micropthalmia) in rat pups[38] (Dawson et al., 1993; Smith et al., 1989, 1992; Johnson et al., 1998a,b; Epstein et al., 1993) given drinking water exposure; increases for both malformations were dose related (Dawson et al., 1993; Smith et al., 1989, 1992). These malformations have not been reported in inhalation studies, though there is a question as to whether the ability of the methods or the number of pups in the studies were adequate to detect these malformations (Barton and Clewell, 2000). The study of Dawson et al. (1993) raises issues for quantitative analysis because of the wide spacing between low and high dose (exposure to TCE was at 1.5 ppm and 1,100 ppm) and whether TCE or one of its metabolites was responsible for the effect reported (Barton and Clewell, 2000).

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[37]*Attributable risk* is the risk or rate difference that may be attributable to the exposure (Rothman, 1986).

[38]Although no battery of animal tests can provide complete assurance in predicting human developmental effects, the similarity in timing and sequencing of events during embryogenesis and organogenesis, particularly cardiogenesis, between humans and rats suggests that the rat is a suitable nonprimate choice for studying developmental effects.

Graeter et al. (2000) reported that gavage exposure to TCE, DCA, or TCA did not produce a statistically significant difference in cardiac malformations in an abstract lacking information on number of animals examined and exposure levels. DCA exposure, however, produced a statistically significantly increased number of external malformations, and both DCA and TCA individually produced lower fetal body weights in Sprague-Dawley rats. TCA also produced a statistically significant lower mean uterine weight in dams compared with vehicle controls.

Recently Boyer (2000) reported TCE exposure in vitro produces a dose-dependent inhibition of mesenchymal cell transformation (a critical event in development of the heart) in progenitors of the valves and septa of the heart, and briefly mentions the findings of a concurrent study that showed altered gene expression in embryo hearts with maternal exposure to drinking water with 110 ppm TCE. The concentration in the in vitro study was in the millimolar (mM) range, comparable to doses of TCE studied in vivo and, because of factors inherent with using an in vitro paradigm, likely to be much less than those administered to the culture medium (Runyan, 2000). However, although direct quantitative extrapolation of in vitro doses to those studied in vivo is problematic, these observations support a TCE effect on cardiac development. Moreover, the finding of cardiac anomalies in rat pups exposed to two TCE metabolites, TCA and DCA, supports a hazard for this effect with TCE (Smith et al., 1989, 1992; Johnson et al., 1998a,b; Epstein et al., 1993).

Both TCA and DCA induce other effects besides cardiac and eye anomalies in offspring. Both TCA and DCA exposure were associated with a substantial increase in implantation and resorption sites[39] and decreased fetal weight (Smith et al., 1989, 1992). These effects were observed in the absence of maternal toxicity.

Exposures to TCE during the prenatal period have been reported to induce neurobehavioral alterations in rat pups, including changes in long-term exploratory and locomotor behavior, altered glucose uptake and metabolism in the neonatal brain, and decreases in myelin (Taylor et al., 1985; Isaacson et al., 1990). In addition, changes in adult behavior are suggested with perinatal exposure to 50 mg/kg TCE (Fredricksson et al., 1993). Statistical analysis of the data used individual pups, possibly resulting in an overestimate of the statistical significance of observed results.

Exposure to TCE can potentially modify responses to other chemical exposures, which is an important consideration because TCE environmental exposure is usually not isolated but also concurrent with other chemicals. TCE and DEHP (a peroxisome proliferator) together were

[39]Implantation sites are sites of initial attachment of a fertilized ovum, but with no further growth. Resorption sites are sites of implantation with embryonic growth, but arrest and necrosis during development.

reported to have synergistic effects with regard to prenatal loss, pup weight changes, and anopthalmia (Narotsky et al., 1995a,b).

3.4.5.2. *Endocrine System Effects and Reproductive Toxicity*

Reports of TCE-induced effects on human sex hormones and their binding proteins are just beginning to emerge in the published literature. For the most part, workplace exposure to TCE has not been reported to change hormones, spermatogenesis, or semen quality in men (Goh et al., 1998). Recently, however, low-level subchronic TCE exposure (mean 30 ppm) was associated with changes in insulin and steroid level, decreased serum testosterone, and decreased sex hormone binding globulin (SHBG[40]) in male workers (Goh et al., 1998). Exposure gradients were observed for several parameters. In analyses adjusted for age, serum testosterone and SHBG were observed to decrease whereas free androgen levels increased with increasing duration of TCE exposure.[41] Moreover, dehydroepiandrosterone (DHEA)[42] increased with years of TCE exposure (Chia et al., 1997), contradicting expectations as DHEA levels normally decrease with age (Metzger et al., 1997; Chiu et al., 1999). A more complicated picture was observed for insulin. Insulin levels were elevated for exposures of less than 2 years, but decreased to below-normal levels for increasing durations of exposure. The normal SHBG/insulin relationship appeared to be disturbed with longer durations of exposure (Goh et al., 1998).

TCE exposure has been shown to affect the reproductive system of males; studies in females are few. In another report of the same population of workers studied above, increase in urinary levels of TCA was correlated with hyperzoospermia, which has been implicated in infertility (Chia et al., 1996). A lower percentage of normal sperm, compared to referent values identified by the World Health Organization, was also observed in this population (Chia et al., 1996). TCE has been shown to accumulate in the male reproductive tract of rats and to decrease testosterone levels (Zenick et al., 1984; Kumar et al., 2000). Kumar (2000) recently reported significant decreases in total epididymal sperm count and motility, with the magnitude of loss correlated with increased duration of exposure. A concurrent increase in total testicular cholesterol and decreases in enzyme activities, which are rate limiting in the biosynthesis of testosterone, was also reported. Of note is the use of young rats (starting weights of 50 g) in this

[40]SHBG has high affinity but low capacity for testosterone and estradiol 17p, and its level may be related to liver toxicity, as it is produced in the liver.

[41]Free androgen is calculated by the division of testosterone levels by SHBG and is that portion free to be biologically active. A decreased index was achieved by differential rates of reduction of SHBG and testosterone.

[42]DHEA is an adrenal steroid associated with insulin resistance, androgenic effects, peroxisome proliferation, and induction of CYP4A (Mayer, 1998, 1999).

subchronic (12 and 24 weeks) study. This report describes results from a preliminary study that indicated TCE-induced decreases in fertility as well. DCA, a metabolite of TCE, is also a testicular toxin to rats and to dogs at exposures as low as 12.5 mg/kg-d (Katz et al., 1981, Cicmanec et al., 1991).

One report exists of increased menstrual disorders in female workers exposed to TCE (Zielinski, 1973).

3.4.5.3. *Cervical, Prostate, and Testicular Cancer*

The human studies provide some limited evidence for increased cancer risk for certain reproductive organs. Cervical cancer, though sparsely reported, is elevated in tier-I incidence studies (RR=2.4, 95% CI=1.2–4.8, N=8) and in tier-III (dry cleaner) mortality studies (RR=1.7, 95% CI=1.5–2.0, N=43) (Wartenberg et al., 2000). The incidence study by Anttila et al. (1995) provided the greatest weight for an association and reported a statistically significant elevation of cervical cancer among those with greatest urinary TCA levels. The comparison with internal controls in Blair et al. (1998) also is suggestive that TCE may be the etiologic agent, and not some other confounder such as socioeconomic class. Prostate cancer incidence and mortality are also elevated in tier-I studies (RR=1.3, 95% CI=1.0-1.6, N=95; RR=1.2, 95% CI=1.0-1.4, N=131).

In an NTP study (NTP, 1988), interstitial cell neoplasms of the testis were reported in Marshall rats exposed to TCE by gavage. Increased incidences are apparent in the other rat strains when counting the animals alive when the first testicular tumor was observed. In another study, (Maltoni et al., 1986, 1988) reported significantly increased incidences of Leydig cell tumors in male rats exposed to TCE by inhalation. Although testicular cancer is often thought of as occurring spontaneously in some strains of rats late in life, the testicular cancers reported for TCE were shown in multiple strains with lower background rates and at earlier times of appearance (see Table 3-4).

DCA at levels of 50 mg/kg-d and higher for 13 weeks produced prostate gland atrophy and testicular changes that were considered dose dependent (Katz et al., 1981).

3.4.5.4. *Pancreatic Cancer*

Pancreatic cancer incidence mortality is statistically significantly elevated in the dry cleaner (tier-III) studies (RR=1.7, 95% CI=1.2–2.6, N=22; RR=1.3, 95% CI=1.0–1.7, N=42), with mixed results in tiers I and II (Wartenberg et al., 2000). Disturbances in insulin levels as noted by Goh et al. (1998) and Kato-Weinstein et al. (1998) and effects on carbohydrate handling may have a role in the induction of effects on the pancreas by TCE in humans. For example, diabetics have an increased risk of pancreatic cancer (Calle, 1998). In experimental animals,

cancer in this organ has not been well studied in the carcinogenic assays carried out to date. Katz et al. (1981) reported that DCA causes pancreatitis in dogs.

3.4.6. Lung

3.4.6.1. *Pulmonary Effects*

There are few human data reporting pulmonary toxicity for TCE (ATSDR, 1997; Barton and Clewell, 2000). Mice appear to be more sensitive than rats to histopathological changes in the lung via inhalation; pulmonary effects are also seen in rats with gavage exposure. Lungs of mice exposed via inhalation to 500 ppm TCE for 30 minutes displayed vacuole formation and endoplasmic reticulum dilation, specifically in Clara cells of the bronchial tree (Villaschi, 1991). Additionally, a reduction in pulmonary CYP450 enzyme activity was observed after a 6-hour exposure to 100 ppm TCE (Odum et al., 1992). TCE administered to rats via gavage at doses 1,000 mg/kg-d and higher caused rales and dyspnea (Narotsky et al., 1995) and pulmonary vasculitis (NTP, 1990).

At relatively high doses (drinking water exposures of 80.5 mM DCA or 45.8 mM TCA), lung inflammation (perivascular) is seen in rats exposed to both these TCE metabolites, but not in controls (Bhat et al., 1991). At lower doses, a secondary effect of DCA exposure in dogs has been reported to be pulmonary inflammation (Katz et al., 1981).

3.4.6.2. *Lung Cancer*

The epidemiologic studies are not generally supportive of lung cancer; lung cancer mortality was statistically significantly elevated only in dry cleaners (tier-III) (RR=1.3, 95% CI: 1.1-1.5, N=137) (Wartenberg et al., 2000). TCE has been reported to significantly increase the incidence of lung tumors in male Swiss mice and female B6C3F1 mice by inhalation (see Table 3-5) (Maltoni et al., 1986, 1988). Another study extended this result to female ICR mice (males were not tested) (Fukuda et al., 1983). These studies did not observe lung tumors in rats. Ingested TCE has not been observed to cause lung tumors in either rats or mice. This is the only tumor site reported to have a positive carcinogenic response by one route but not another.

3.4.7. Genetic Toxicity

TCE and a number of its metabolites (CH, DCA, and TCA) have been evaluated in a large number of genotoxic assays.[43] On the basis of the available data, definitive conclusions cannot be drawn as to whether TCE is or is not a genotoxic carcinogen. Taken together, TCE and its metabolites CH, DCA, and TCA are, at most, very poor genotoxicants. There is evidence that the metabolite DCVC is more potent as a potential genotoxic agent. Evidence from a number of different analyses and a number of different laboratories using a fairly complete array of genetic endpoints indicates that TCE may have some potential to be genotoxic. However, the evidence is not convincing that it is genotoxic, and is confounded by the fact that TCE is often stabilized with a very low concentration of epichlorohydrin or 1,2-epoxybutane, both known to be potent mutagens. A series of carefully controlled studies evaluating TCE (without mutagenic stabilizers) found TCE incapable of inducing point mutations in standard *Salmonella* assays. Furthermore, in vitro gross chromosome aberration assays were negative. In vivo, there is some evidence that TCE or its metabolites bind to DNA and can induce single-strand DNA breaks in both hepatic and kidney cells. However, the dose required to cause these DNA breaks was very high (4-10 mM of TCE/kg body weight) and the response was very low. More recent data show TCE to affect DNA methylation in whole-liver preparations at lower exposures with increased expression of the two proto-oncogenes *c-jun* and *c-myc* (Tao et al., 1999). Moore and Harrington-Brock's review of the potential genotoxicity of TCE (Moore and Harrington-Brock, 2000) notes that TCE is negative in a number of standard tests, and furthermore, in those tests where positive responses were observed, the dose was very high and response low. Accordingly, although it is not possible to totally eliminate the possibility that TCE would be a genotoxic carcinogen, it is clear from these evaluations that TCE is not a potent genotoxicant (Moore and Harrington-Brock, 2000).

CH has been extensively studied as a potentially genotoxic agent. It has been evaluated in the recommended battery[44] and several other assays, including genetic alterations in rodent germ cells. CH is weakly positive at very high concentrations in in vitro genotoxicity assays that detect point mutations, chromosomal mutations, and/or aneuploidy. There is a mixture of positive and negative in vivo data, and because there is no reason to weigh any of the studies

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[43]Genetic toxicity can be defined using mutational endpoints, cytogenetic analysis, and evaluation of primary DNA damage with mutagenicity described as the ability to induce heritable mutations (Moore and Harrington-Brock, 2000). Primary DNA damage may occur via adduct formation, strand or chromosomal breakage, and unscheduled DNA synthesis. Mutational damage consists of point mutations, chromosomal rearrangement, deletions, loss or gain of chromosomes (aneuploidy), or changes in whole chromosome complements (polyploidy) (Moore and Harrington-Brock, 2000).

[44]The core battery of recommended tests for genetic toxicity include the *Salmonella* assay, in vitro mouse lymphoma mutation assay, and in vivo cytogenetic assay (Moore and Harrington-Brock, 2000).

more than the others, it is not clear whether CH has the potential to be genotoxic in vivo (Moore and Harrington-Brock, 2000).

DCA and TCA have also been extensively studied using a variety of genotoxicity assays, including the recommended battery. DCA is genotoxic at very high concentrations in the *Salmonella* assay, the in vitro mouse lymphoma assay, and in vivo cytogenetic and gene mutation assays. DCA can cause DNA strand breaks in mouse and rat liver cells following in vivo administration by gavage. As with TCE and CH, the concentration of DCA required to induce damage is very high and the level of response is generally very low. Therefore, although one cannot eliminate the possibility that DCA might induce tumors via a mutagenic mode of action, the weight of the evidence argues that TCE-induced tumors would not be mediated by DCA-induced mutation. TCA is the least mutagenic of the TCE metabolites discussed so far. It is negative in the Salmonella assay, and although positive in the mouse lymphoma assay, it is substantially less potent than DCA or CH. It is unclear whether TCA can induce chromosomal damage in vivo; some assays are positive and some negative. TCOH is negative in the *Salmonella* assay but has not been evaluated in the other recommended screening assays. Therefore, it is unclear whether TCOH is genotoxic (Moore and Harrington-Brock, 2000).

Cysteine intermediates (DCVC and DCVG) formed by the GST pathway are capable of inducing point mutations, as evidenced by the fact that they are positive in the *Salmonella* assay. DCVC is the most potent of the TCE metabolites as a *Salmonella* mutagen; DCVG appears to be similar in potency to DCA. Although DCVC and DCVG have not been evaluated in the other EPA-recommended screening assays, there is some indication that they can induce primary DNA damage in mammalian cells in vitro and in vivo. Long-term DCVC exposure can induce de-differentiation of LLC-PK1 cell clones, supporting evidence of genetic toxicity (Vamavakas et al., 1996). DCVC has also been shown to induce the expression to two proto-oncogenes, *c-fos* and *c-myc* (Lash et al., 2000b). Moreover, chlorothioketenes, which are the intermediates formed from the further metabolism of DCVC by $-lyase, have been shown in vitro to react with DNA (Müller et al., 1998a,b) and, in an aqueous solution, to bind with cytosine (Volkel et al., 1998).

3.5. MODES OF ACTION OF TCE TOXICITY

The previous section identifies TCE and its metabolites as eliciting toxicity in a number of organs in both humans and laboratory animals. Possible modes of action for these observed effects are discussed in this section, which focuses primarily on events occurring in the rodent so as to put into perspective observations in chronic toxicity assays. A discussion of the human relevance of these possible modes of action is also presented. Much of the discussion focuses on events that may give rise to rodent liver, kidney, and lung tumors. The epidemiologic analysis, however, suggests other organ systems may also be targets. Moreover, noncancer toxicity is

observed and may arise through a common mode of action with the carcinogenic effects. Mode-of-action hypotheses for these observations are presented last.

An understanding of how TCE may elicit toxicity in a number of organ systems can arise through the identification of very specific events such as changes in gene regulation and cell signaling, or through a discussion of more generalized events such as common changes to physiological processes. Data supporting mode-of-action hypotheses are not uniform across potential targets of toxicity. Identifying mode(s) of action for TCE toxicity entails obtaining answers to several key questions concerning the relative amount of metabolites formed between species, the ability to measure active metabolites in biological samples (e.g., DCA), the determination of which metabolites may impart adverse effects, the sequence of biological changes that can lead to tumor development, and the extrapolation of these events from rodents to humans and from higher level exposure in the bioassay to lower environmental exposure.

The carcinogenicity data on TCE are complex in that a consistent site-specific response is not seen across species. Rather, carcinogenicity is demonstrated in the mouse and the rat, but in different organs. At the most basic level, the observation of rodent tumors indicates a potential human cancer hazard, and the epidemiologic data are further suggestive of a cancer hazard. Mode-of-action data have been generated to explain liver tumor development in the mouse and are rich compared with observed effects in other organ systems; however, much uncertainty still exists. In all cases, key pieces of information are still missing. The similarity between carcinogenic effects induced by parent compound and metabolites supports the conclusion that TCE metabolites are mostly responsible for the liver and kidney tumors observed in TCE bioassays. Accordingly, much of the mechanistic data on the liver are derived from experiments on the oxidative metabolites, TCA and DCA; parallel studies in the parent compound are few, making it difficult to confidently assign the mode of action to a particular metabolite. The complexity of TCE metabolism and clearance also complicate the identification of a metabolite(s) that can be identified as responsible for TCE-induced effects. Not surprisingly, more than one mode of action may explain TCE-induced carcinogenicity and several hypotheses are discussed. In all likelihood, a number of events will be significant to tumor development in the rodent under bioassay conditions. Uncertainty exists, however, as to which events may be more relevant to human exposure to TCE at environmental levels.

3.5.1. Liver Cancer

Much attention has focused on understanding the development of liver tumors in the TCE bioassay and the role of the oxidative metabolites, CH, DCA, and TCA. Each of these metabolites has induced liver tumors in mice under bioassay conditions; DCA also induces liver tumors in the rat. Mechanistic studies have concentrated on TCA and DCA. One of the largest

uncertainties associated with mode-of-action hypotheses based on these metabolites is the shortage of parallel evidence with TCE itself. Little experimental evidence is available on CH, and its rapid metabolism to the haloacetic acids is believed to minimize a direct role of CH in TCE-induced liver cancer (Bull, 2000). Several hypotheses have been put forth regarding the development of liver tumors in the mouse. These include the role of the peroxisome proliferator-activated receptor (PPAR) and peroxisome proliferation, disturbances in cell signaling, and effects on DNA (Bull, 2000). A short discussion of how these events are consistent with other physiologic conditions associated with hepatocarcinogenicity is presented at the end of the section.

3.5.1.1. *Peroxisome Proliferation*

The prevailing view of TCE-induced mouse liver carcinogenesis has been that these tumors arise in parallel with peroxisome proliferation in the liver by TCE metabolites (Elcombe, 1985; Elcombe et al., 1985; Goldsworthy and Popp, 1987; Melnick et al., 1987; DeAngelo et al., 1989; Cattley et al., 1998). Specifically, TCA is most closely associated with this hypothesized mode of action because a sustained response with chronic exposure has been noted, in contrast to the brief response reported for DCA (Bull, 2000). How tumors may develop from peroxisome proliferation remains unclear (Bull, 2000; Zhou and Waxman, 1998). It has been proposed that oxidative damage caused by marked increases in free-radical generating enzymes and peroxisomal $-oxidation might initiate carcinogenesis. It was generally believed that as peroxisome proliferation has not been observed in humans, agents that produced this result in the rodent would not present a carcinogenic hazard to humans.

This view had merit in the past; however, emerging data on the peroxisome proliferator-activated receptor (PPAR) implicate nonperoxisomal events such as PPAR-triggered gene transcription as having important bearing on liver toxicity and subsequent tumor development. This position is consistent with the view taken by an expert panel convened by ILSI, which concluded that the ability to act as a peroxisome proliferator does not exclude the possibility that other properties independently contribute to the development of cancer (Cattley et al., 1998; Bull, 2000). Moreover, this expert panel determined that there was insufficient basis for concluding that these compounds did not represent a carcinogenic hazard to humans. More recent experimental evidence suggests that chemicals that induce peroxisome proliferation also produce a carcinogenic response in rodents through the activation of the PPAR by the chemical or its metabolite(s). The activated PPAR will interact with the retinoid-X receptor (RXR) and other specific response elements, resulting in increased synthesis of peroxisomal enzymes and promotion of gene transcription including transcription of enzymes important in lipid metabolism.

The PPAR belongs to the steroid hormone receptor superfamily and includes several distinct types: PPAR(, PPAR*, and PPAR". PPAR" is found in many species including mice, rats, and humans. It has been mostly identified in the liver of rodents and more recently in the human liver. PPAR" is also found in other human tissues including muscle, liver, kidney, heart, and testis (Youseff and Badr, 1999; Schultz et al., 1999). Human liver PPAR" mRNA has been reported to be 10-fold lower than that of mouse liver, whereas that of skeletal muscle is higher in humans than in rats (Youseff and Badr, 1999). Interindividual variations are also evident (Tugwood et al., 1998).

TCE and its metabolites have been shown to induce peroxisome proliferation in rodents and its metabolites (TCA and DCA) to activate PPAR" (Goldsworthy and Popp, 1987; Maloney and Waxman, 1999; Zhou and Waxman, 1998). However, TCE, TCA, and DCA are considered as weak peroxisome proliferators compared to the model drug, WY-14643. Recent in vitro experimental evidence at high concentrations of both TCA and DCA shows activation of human PPAR" (Maloney and Waxman, 1999). No species differences were noted in the magnitude of activation, which, for both human and mouse PPAR" activation, appeared to increase with increasing concentration of either TCA or DCA. High concentrations of TCA, but not DCA, were further shown to activate PPAR(from mice; no activity was demonstrated for human PPAR((Maloney and Waxman, 1999).

PPAR activation is likely more important as a possible mode of action for TCA than for DCA, even though both DCA and TCA are shown to activate mice and human PPAR". As discussed more thoroughly in the next section, TCA- and DCA-induced tumors are characteristically very different. These observations support an inference of differing modes of action for tumor induction by each of these metabolites. PPAR" activation is seen in vitro with TCA concentrations in mM range (Issemann and Green, 1990; Maloney and Waxman, 1999), approximately the same range that would be reached in blood of mice by treatment with TCE at the doses employed in the in vivo cancer bioassays (Bull, 2000). Like TCA, mM concentrations of DCA are also shown to activate PPAR". However, in contrast to TCA, systemic concentrations of DCA associated with experimental doses that induce liver tumors in mice are expected to be very small (Barton et al., 1999). Moreover, apoptosis and other effects seen with DCA exposure (discussed in the following section) are expected to occur at DCA concentrations much lower than the in vitro concentrations producing PPAR activation. Thus, PPAR activation and the resulting pleotrophic responses associated with receptor activation are not expected to contribute greatly to DCA's carcinogenic mode of action.

TCA's ability to activate PPAR" has cross-species relevance. The observations of Maloney and Waxman (1999) support a qualitative similarity between mice and humans; thus, a PPAR hypothesized mode of action is relevant to both mice and humans. As identified above,

quantitative differences in the expression of PPAR" exist between mice and humans, with humans in general having a lower expression of PPAR" compared to mice. These quantitative differences have import to the dose-response analysis of the mouse liver tumors and will be more rigorously treated in Section 4, Dose-Response Assessment and Characterization.

3.5.1.2. *Cell Signaling and Effect of Negative Selection and Apoptosis*

Bull (2000) suggests that modification of cell signal pathways by TCA and DCA resulting in alterations in cell replication, selection, and apoptosis (programmed cell death) are likely important contributions to the hepatocarcinogenicity of TCE and its metabolites. TCA's ability to activate PPAR and the subsequent cascade of pleotrophic responses, including effects on gene transcription, is a classic example of cell signaling. DCA exposure has additionally been shown to influence other cell signaling pathways, and observed perturbations provide insight on mode-of-action hypotheses regarding induction of DCA tumors.

Hepatomegaly, or increase in liver size, is a common feature of TCE, DCA, TCA, and CH exposure to rodents. Hepatomegaly associated with TCE is observed at experimental exposures well below those shown to induce liver tumors in mice (Bull, 2000). Hepatomegaly is influenced by increases in either cell number or cell size. Cell number is affected by cell replication rates and by rates of apoptosis. There is also some evidence that hepatomegaly associated with PPAR" activation results from suppressed apoptosis (Roberts et al., 1995); Kupffer cells have been shown to contribute to this process (Chevalier and Roberts, 1998; Rose 1997). One of the roles of apoptosis appears to be the prevention of damaged cells from dividing and progressing to tumors.

Hepatomegaly seen with TCA and DCA exposure is engendered by different processes. Hepatomegaly associated with DCA is attributable to cytomegaly, increases in cell size, rather than increases in cell numbers (Bull, 2000). Hepatocytes stain heavily for glycogen, which accumulates in cells within 1 to 2 weeks of treatment and becomes more severe over time (Bull, 2000). These observations are quite different from the hepatomegaly induced by TCA. For TCA, increases in liver weight are observed soon after exposure and are linear with experimental dose (Bull, 2000); however, this is primarily a reflection of cell number and not cell size.

Experimental observations support the inference that DCA and TCA induce tumors that vary in growth rate and develop from different clones of cells in mouse liver (Bull, 2000). Tumors produced from DCA and TCA exposure display different characteristics. DCA produces more benign foci that lead to small adenomas, although larger lesions include carcinomas. TCA elicits fewer altered foci but more carcinomas. These two metabolites, moreover, show divergent responses once exposure is terminated (Bull, 2000). Termination of DCA treatment halts progression of the foci and adenomas to carcinomas. In contrast, carcinomas are still observed

after termination of TCA exposure, although fewer in number (Pereira and Phelps, 1996). Differences are also reported in the immunoreactivity of tumors induced by TCA and DCA in regard to the proto-oncogenes *c-jun* and *c-fos* (Bull, 2000). DCA- and TCA-induced tumors also appear to have differences as to the shape of their respective dose-response curves, with the number of DCA-induced tumors appearing to increase disproportionately with dose while those of TCA appear to be linear.

One possible explanation for these observations is that DCA and TCA may selectively modify growth rates of different clones of cells. TCE and its metabolites could alter cell replication and apoptosis rates in altered hepatic foci and preneoplastic foci, and provide a selective growth advantage for differing clones of cells rather than a generalized proliferative response. Cells resistant to the downregulation of mitosis induced by a chemical may have selective growth advantage over other cells in that organ (Bull, 2000).

Effects of DCA and TCA on replication rates in foci and normal hepatocytes vary and appear to be dose dependent in some cases. DCA at higher doses can increase replication rates of tumor cells (Bull, 2000), whereas only a small stimulatory effect on replication is seen in extrafocal (normal) hepatocytes. Further, as treatment progresses, an inhibitory effect is observed in the extrafocal hepatocytes. This suppression of cell replication in extrafocal hepatocytes is accompanied by a parallel decrease in apoptosis. These observations raise a question as to which is the driver of the decreased cell turnover in extrafocal hepatocytes seen with DCA. It is unknown if the decrease in cell turnover would increase the probability of transformation of hepatocytes to allow clonal expansion of damaged hepatocytes that would normally be eliminated (Bull, 2000). This possibility is suggested by the modeling of DCA lesion growth rates by Miller et al. (2000).

TCA depresses replication rates in normal hepatocytes with no indication that replication rate within tumors are modified by TCA treatment (Bull, 2000). TCA appears to be selecting initiated cells that are more aggressive than those selected by DCA at low doses. The extent to which replication rates in extrafocal hepatocytes are decreased with longer term TCE exposure has not been investigated, nor those in foci or tumors.

Exposure to DCA has been shown to perturb a number of normal cellular functions including cell signaling pathways, particularly those associated with carbohydrate handling (Bull, 2000). DCA has more recently been shown to alter serum insulin concentrations in mice (Kato-Weinstein et al., 1998). Insulin is recognized to be mitogenic in liver (Bull, 2000) and to have differing effects on apoptosis (Hermann et al., 1999). Further, insulin receptor expression in extrafocal portions of the liver is suppressed by DCA (Bull, 2000). The principal intracellular substrate of the insulin receptor, tyrosine kinase, is linked to enzymes regulating glycogen synthesis. In fact, DCA exposure to mice inhibits the glycogen synthesis enzymes and is also

associated with increased glycogen deposition in extrafocal hepatocytes, but not in preneoplastic foci, which are glycogen poor. DCA can also inhibit pyruvate dehydrogenase kinase, although at levels higher than those needed for effects on glycogen storage. The dose-response for glycogen deposition in the liver is in the same range as that seen with liver tumors, raising questions of whether modification of a same-cell signaling pathway may be responsible for both observations. Moreover, the accumulation of glycogen in normal hepatocytes may contribute to the differential effects of DCA treatment on insulin receptor expressed in extrafocal hepatocytes and those in altered foci (Bull, 2000). DCA thus may alter the insulin signaling pathway and provide a growth advantage to initiated cells through a signal transduction pathway necessary for tumor growth (Kato-Weinstein et al., 1998). The precise nature of this pathway, however, has yet to be fully elucidated.

Further, a number of metabolic disturbances are associated with exposure to DCA. A major site of action of DCA at high doses is pyruvate dehydrogenase (PDH), the rate-limiting enzyme of glucose oxidation (Stacpoole et al., 1992). DCA exposure has been shown to inhibit hepatic triglyceride and cholesterol biosynthesis, whereas the stimulation of PDH results in effects on gluconeogenesis (Stacpoole et al., 1992). Emerging experimental evidence, moreover, suggests that DCA may interfere with tyrosine catabolism. DCA's further metabolism is dependent on a glutathione-S-transferase isozyme, GST zeta (GSTZ) (Anderson et al., 1999), also identified as maleylacetoacetate isomerase (Fernández-Cañón et al., 1998). Maleylacetoacetate isomerase is one step in the catabolism of tyrosine. Chronic exposure to DCA causes an irreversible inactivation of GSTZ (Anderson et al., 1999), with the rate of inactivation greater for rats than for humans; the rate of inactivation in the mouse falls in between those for the rat and human (Tzeng et al., 2000).

The interrelationship between rates of cell replication with rates of cell deaths make it very difficult to determine if the effects of TCA and DCA are exerted on one process or the other (Bull, 2000). Although the mechanisms underlying DCA- and TCA-induced alterations in cell division and death are not yet known, the experimental observations suggest that each metabolite differentially alters these processes. However, the patterns of effects on the processes of cell division and death provide a reasonable framework for TCE-induced liver cancer. Moreover, although it is not yet clear whether DCA-induced effects on carbohydrate handling and on other metabolic processes are directly related to cancer induction, such effects are consistent with a hypothesis regarding selection and can be consistent with other physiologic conditions associated with hepatocarcinogenicity.

3.5.1.3. *Effects on DNA*

A key question to risk assessment is whether TCE-induced tumors are mediated through an induced mutational mechanism (Moore and Harrington-Brock, 2000). Examination of tumors for possible mutation has been carried out for investigation of this potential mode of action. TCE and its metabolites CH, DCA, and TCA are, at best, very poor genotoxicants (Moore and Harrington-Brock, 2000). TCE and DCA at high concentrations have been shown to bind to DNA and proteins, and to induce single-strand breaks (Moore and Harrington-Brock, 2000; Bull, 2000). The magnitude of response, however, is low compared to other more potent genotoxicants such as MMS and DMN (Moore and Harrington-Brock, 2000). DCA is genotoxic and has been shown to cause DNA strand breaks in mouse and rat liver cells following high level in vivo gavage exposure. TCA is the least mutagenic of the TCE metabolites (Moore and Harrington-Brock, 2000).

Mutation frequency and mutation spectra in the *ras* oncogene have been examined in TCE-, TCA-, and DCA-induced liver tumors from male mice; it is interesting to note that very few codon 61 *h-ras* mutations are seen in liver tumors from female mice treated with DCA. The actual amount of mode-of-action information on induced mutational mechanisms from these data is quite minimal (Moore and Harrington-Brock, 2000). However, the pattern of mutation seen helps provide insight into the relative contribution of different metabolites to TCE-induced liver cancer (Bull, 2000). Mutational frequency in DCA and TCE tumors (arising from drinking water exposure) does not differ significantly from that observed in spontaneous tumors; TCA tumors show both a higher mutation frequency and a pattern atypical of that found with other peroxisome proliferators (Bull, 2000; Maronpot et al., 1995). Comparison of nucleotide base sequences within codon 61 of *h-ras* shows some differences between spontaneous tumors and DCA and TCE tumors, suggesting a similarity between tumors from DCA and TCE-treated animals (Moore and Harrington-Brock, 2000). Route of exposure, however, appears important to mutational frequency associated with TCE-induced liver tumors (Bull, 2000).

The similarity between DCA and TCE tumors in terms of frequency of mutation and its spectra support an inference that DCA contributes an important role in the hepatocarcinogenicity of TCE. The theoretical modeling of TCE, DCA, and TCA liver tumors of Chen (2000) provides further support for DCA's contribution to TCE-induced liver cancer, although a role for TCA cannot be excluded either. As additional supportive evidence, lower doses of DCA that accompany higher doses of TCA appear to exert a large influence on tumor development. Female mice initiated with N-methyl-N-nitrosourea followed by promotion with a mixture containing both DCA and TCA developed tumors that appeared more similar to tumors promoted by DCA alone than those by TCA alone (Pereira et al., 1997). Moreover, the number of tumors produced from the mixture was greater than would be achieved by adding together tumor incidences for the individual exposures.

The interaction of TCE and its metabolites to bind directly with DNA may not be as important at lower exposure levels as effects on gene expression. Specifically, effects on gene expression, whether through activation of PPAR or other signal pathways, may be more important to their potential carcinogenic properties than induced mutation. Moreover, effects such as the mistargeting of covalent modification to the proteins that package DNA (e.g., histone acetylation) or to DNA itself (e.g., cytosine methylation) may be involved in cancer induction, not by altering gene sequence, but by modifying transcription of the gene (Wolffe, 1999). For example, DNA methylation is believed to play a role in the control of gene transcription (Goodman and Counts, 1993; Counts and Goodman, 1995).

TCE and its oxidative metabolites have been shown recently to decrease methylation of DNA as 5-methylcytosine (5MeC) in addition to demethylation of the promoter regions of two proto-oncogenes, *c-jun* and *c-myc* (Tao et al., 1998, 1999, 2000a). Moreover, the decreased methylation and increased expression of *c-jun* and *c-myc* with TCA and DCA exposures also occurred in the presence of increased DNA methyltransferase activity (Tao et al., 2000b). The proto-oncogenes, *c-jun* and *c-myc*, participate in the control of cell proliferation and apoptosis (Tao et al., 2000a). The expression of mRNA for these two genes was shown to be transiently increased as well (Tao et al., 1999). This is an early observation, seen after 3 days of exposure to TCE. In fact, cell proliferation is enhanced during the first few weeks of exposure to DCA and TCA (Bull, 2000). Hypomethylation associated with exposure to DCA appears to be reversed once exposure is terminated (Tao et al., 1998), in contrast to the extent of hypomethylation associated with exposure to TCA, which remained elevated upon exposure cessation. These observations support the hypothesis that DCA, and not some other process or event, is affecting methylation in the tumors. Tao (2000a) recently suggested that depletion of S-adenosylmethionine may be responsible for the observed hypomethylation of DNA. The prevention of DCA-, TCA-, and TCE-induced DNA hypomethylation by the addition of methionine supports this hypothesis.

The relationship between DNA methylation and cancer induction is not well elucidated, and the events and their sequence, which are important to neoplastic cell transformation, are lacking. Consequently, at the current time, DNA methylation may be best considered an effect of exposure rather than a precursor specific to the development of tumors.

3.5.1.4. *Evaluating Proposed Modes of Action for Liver Carcinogenesis*

There are several important questions that arise from the discussion above: which of the hypothesized modes of action can be judged as more plausible; is there evidence to reason any one is the mode of action for TCE hepatocarcinogenicity, and if so, is it important to EPA's proposed cancer guidelines (U.S. EPA, 1999); whether an event considered as a precursor to

carcinogenesis can be identified. Hence, insight into these questions can support weight-of-evidence determinations and approaches that will be used in the quantitative dose-response assessment presented in Section 4. EPA's proposed cancer guidelines (U.S. EPA, 1996, 1999) note the necessity to outline the sequence of events leading to cancer, to identify key events that can be measured, and to weight information to determine whether there is a causal relationship between events and cancer formation. The validation of a causal relationship between exposure and cancer risk includes the consideration of a number of factors, many of which have been borrowed from the epidemiologic literature. These criteria include (1) consideration of the strength, consistency, and specificity of the association between event and tumor response; (2) the presence of a dose-response relationship between the key event and tumor endpoint; (3) the temporal relationship between tumor onset and occurrence of the key event (i.e., the key event should precede tumor formation); (4) the biological plausibility and coherence of the mode of action; and (5) consideration of other modes of action. Experimental evidence that can be used to address these criteria is often derived in the species in which tumors were observed. However, the ultimate question is the human relevance of the proposed mode of action. Hence, it is necessary to demonstrate that the sequence of steps leading to tumorigenesis in the rodents will also occur in humans.

Experimental evidence for support of mode-of-action hypotheses in the liver focuses on two of TCE's oxidative metabolites, TCA and DCA. Few data are available on TCE exposure directly, and only in studies involving activation of the PPAR, albeit in vitro, experiments of human hepatic cells. Accordingly, it is difficult to determine which of the metabolites of TCE may be responsible for its toxicity, what the key events may be, and what is their relevance to humans. There is enough evidence, however, to suggest that many of the responses to TCE metabolites are relevant to the ability of TCE to induce cancer of the liver in animals, as well as to the extrapolation of these observations to humans.

TCA and DCA have been shown to induce a number of different events in livers of mice (discussed in the preceding sections). Both have been shown to activate PPAR" as evidenced by peroxisomal proliferation; however, a role of PPAR activation as a possible mode of action is more plausible for TCA than for DCA. TCA induces a more sustained proliferative response than DCA. Furthermore, DCA induces effects on cell signaling processes and on carbohydrate handling at lower concentrations than those associated with peroxisome proliferation. Besides PPAR", TCA has been shown to activate PPAR(from mice but not humans.

The role of PPAR" activation and the sequence of events following activation that are important to tumorigenesis are not well defined, nor is the contribution of other forms of PPAR to observed TCE carcinogenicity currently known. Furthermore, extraperoxisomal effects of activation of transcription by PPAR" may elicit a number of changes in cell signaling that may

also be linked to increased cancer risk. Moreover, key events critical to the induction of tumors and the cross-species relevance of these key events have yet to be identified. Further investigations regarding activation of PPAR through the use of genetically altered experimental animals may provide insight regarding the role of PPAR" in the induction of liver tumors by TCE and the contribution of TCA formation.

DCA has the ability to affect other cell signaling processes and to disturb carbohydrate handling at lower concentrations than those associated with PPAR activation. Specifically, DCA has been shown to alter cell replication, selection, and apoptosis. The observed disturbances to insulin signaling and to carbohydrate handling associated with DCA exposure may also be connected to these alterations. The events that lead to and result in a selective growth advantage or in depressed apoptosis of altered hepatic foci have yet to be clearly defined. As with activation of PPAR, critical events important to the induction of liver tumors in mice administered DCA have yet to be identified.

Another potential mode-of-action hypothesis of DCA liver tumor induction recently put forth by Cornett et al. (1999) involves disturbance of tyrosine catabolism. DCA's inhibitory effects on GSTZ may also inhibit maleylacetoacetate isomerase. Inhibition of maleylacetoacetate isomerase activity could consequently lead to a buildup of intermediates of tyrosine catabolism, several of which have are considered alkylating agents (Cornett et al., 1999). This proposed mode of action needs further investigation because direct experimental evidence regarding TCE-induced effects on tyrosine intermediates such as maleylacetoacetate and maleylacetone is lacking. At the present time, the hypothesis of DCA-induced effects on cell signaling is more compelling.

Another approach for investigating the possible role of TCE metabolites in its toxicity is to ascertain whether enough of the metabolite is present after administration of the parent compound to induce tumors at the target of concern. A critical issue is the amount of DCA produced from chronic exposure to TCE and whether this amount would be sufficient to elicit the events that have been observed with experimental administration of DCA. Chronic exposure to DCA has been shown to increase the elimination half-life of subsequent exposures in mice, rats, and humans, suggesting that chronic exposure to DCA inhibits its own metabolism (Gonzales-Leon et al., 1999). Moreover, recent reports of irreversible inactivation of DCA by GSTZ suggest larger internal doses of DCA may occur than previously assumed (Tong et al., 1998; Tzeng et al., 2000). Thus, factors that may affect the clearance of DCA and the role of a possible buildup of toxic metabolites are complex and are another uncertainty associated with this assessment.

Nevertheless, Barton et al. (1999) have examined this question for mice by carrying out a theoretical modeling study of both TCE and DCA pharmacokinetics. This study estimated that

the maximum levels of DCA in the serum produced from 1,000 and 2,000 mg/kg gavage doses of TCE (tumorigenic doses in the bioassay) would be equivalent to internal dose metrics that were approximately half of those estimated from exposure to 0.5 mg/L DCA in drinking water. Exposure of mice to 0.5 g/L DCA in drinking water produced a 48% to 63% incidence of hepatocellular carcinomas and a 75% incidence of combined liver tumors (carcinomas and adenomas), with an increase in the number of carcinomas per liver compared with livers from control animals (Daniel et al., 1992; DeAngelo et al., 1999; Bull, 2000). The analysis of Chen (2000) provides further support that DCA produced by TCE is capable of producing the carcinogenic response seen in mice from gavage exposure to TCE.

Information needed to extrapolate the potential risk of DCA formation from TCE exposure in regard to human liver cancer induction is more limited. Unfortunately, Barton et al., (1999) do not examine the amount of DCA produced in humans from TCE exposure, and only one study (Fisher, 1998) is available that assayed DCA in serum from 17 human volunteers (9 males and 8 females) exposed via inhalation for 4 hours to 100 ppm TCE. Fisher (1998) noted low but detectable levels of DCA in serum in five volunteers (three males and two females) with levels ranging between 4 and 12 ppm. The lack of detection of DCA in the other 12 subjects may be due to the rapid metabolism of DCA, as inhibition of DCA's metabolism would not be expected to occur from a 4-hour exposure. Alternatively, but less likely, the absence of DCA in these human subjects may be from lack of DCA formation from TCE. Only one subject with detectable DCA levels in serum was exposed to TCE at the lower level of 50 ppm for 4 hours, and DCA was not detected in this experimental protocol (Fisher, 1998, personal communication with J. Fisher). Furthermore, DCA has been detected in children and adults who received chloral hydrate (Henderson et al., 1997; Yan, 1999). These studies together provide support that humans, like mice, have the potential to produce DCA from either TCE when administered as parent or from a key TCE metabolite, chloral hydrate.

The technical problems with detection of DCA, however, are difficult, particularly in the presence of large amounts of trichloroacetic acid, and samples are subject to the presence of DCA as an artifact (Ketcha et al., 1996). Hence, it is not clear whether the reported findings of DCA in humans are accurate or are a reflection of conversion of TCA to DCA under acidic conditions. The more recent data of Gonzalez-Leon (1999) and analysis of Barton et al., (1999) are not subject to analytical artifact; thus, it can be concluded that mice convert DCA from TCE. Given the large uncertainty associated with the human data, by default, it is reasonable to assume that humans would similarly produce DCA and observations in mice can be extrapolated across species to humans.

Several lines of reasoning lead to the conclusion that no one metabolite can be identified as responsible for TCE's carcinogenicity. The modeling studies of Barton et al. (1999) and Chen

(2000) support an inference that the concentrations of DCA in mice produced from exposures experienced in the TCE gavage bioassay are of sufficient quantity to induce hepatic tumors. Conclusions of Chen (2000) further suggest ambiguity in identifying one metabolite as explaining TCE hepatocarcinogenicity. Both metabolites may each have their separate modes of action and may, in fact, work in concert to alter each other's toxicity. This is suggested from the experimental evidence of joint TCA and DCA exposure by Pereira et al. (1997) and from newly emerging experimental data of Bull *(personal communication).* Bull (personal communication) has examined the frequency of TCE-induced liver tumors that are *c-jun+*; the frequency of hepatic tumors in which *c-jun* has been mutated is high in DCA-induced liver tumors. Preliminary results from Bull suggest a role for DCA, in that many TCE-induced tumors were *c-jun+* (Bull, personal communication). Also noteworthy was a finding of a similar number of tumors that did not display a mutation to *c-jun* (Bull, personal communication); *c-jun* is not mutated in liver tumors from TCA exposure. These findings suggest that TCA and DCA both contribute to the hepatocarcinogenicity of TCE.

3.5.1.5. *Consistency With Other Physiologic Conditions and Associated Hepatocarcinogenicity*

The consistency in events seen with exposure to TCE metabolites, specifically DCA, and physiologic conditions associated with several human diseases can provide insight into mode-of-action hypotheses and may support inferences regarding potential increased human cancer risks. Many cellular events observed with exposure to DCA, including changes in carbohydrate handling, are also seen with several diseases such as glycogen storage diseases and diabetes. Although the pathogenesis of these diseases and what is known about liver tumor induction with DCA exposure may have distinct differences, disturbances in cell metabolism and in carbohydrate handling are common events. As discussed below, diabetics and individuals with glycogen storage diseases are at increased risk for liver cancer. Furthermore, individuals with tyrosinemia also have an increased risk of liver cancer (Tanguay et al., 1996; Laberge et al., 1986).

Glycogen storage diseases are caused by inherited deficiencies of enzymes that regulate the synthesis, transport, or degradation of glycogen. Individuals with glycogen storage diseases store excess glycogen in their livers. This storage resembles that seen in the extrafocal hepatocytes of DCA-treated livers. The majority of individuals with glycogen storage diseases develop liver cancer early in life. In fact, multiple hepatocellular neoplasms are often seen in these individuals. In a similar pattern, DCA exposure induces multiple foci in mice livers.

Diabetes is a disease involving insulin disturbances. Poorly controlled diabetes results in excess glycogen storage in livers as well. Hepatomegaly is seen in both Type I and Type II

diabetics (Herrman et al., 1999) and diabetics have an increased risk for liver cancer (LaVecchia et al., 1994; Adami et al., 1996; Wideroff et al., 1997). It is possible that greater sensitivity of tumor cells than normal cells to insulin could mimic such disturbance and contribute to DCA liver tumor induction as well (Bull, 2000).

Moreover, observations on cellular changes that include markers in liver tumors resulting from exposure to TCE, DCA, or TCA overall are consistent with a more generalized description of hepatocarcinogenesis proposed by Bannasch and others (Bannasch et al., 1986, 1996, 1997; Su et al., 1997). A number of findings have been reported that suggest the sequence of cellular changes during hepatocarcinogenesis is in principle identical in humans and experimental animals (Bannasch et al., 1986, 1996, 1997). For example, Bannasch (1986) notes a correlation between changes in cell metabolism, carbohydrate handling, and neoplastic cell transformation.

3.5.2. Kidney Cancer

Mode-of-action studies investigating kidney tumors induced in gavage and inhalation bioassays have focused on metabolites, particularly those associated with the glutathione pathway, where the experimental evidence is more persuasive. There are several lines of investigation for potential modes of action for TCE in the kidney as cited by Lash et al. (2000b): peroxisome proliferation, alpha-2u globulin nephropathy, mutagenicity or other genetic toxicity, formic acid formation, and cytotoxicity-induced damage. The question is which, if any, of these mechanisms are operative and likely to lead to the development of cancer at doses that were lower than those used in the bioassays of TCE in animals.

3.5.2.1. *Genotoxicity*

Cysteine intermediates (DCVC and DCVG) formed in the GST pathway are capable of inducing point mutations, as evidenced from positive findings in the *Salmonella* assay (Moore and Harrington-Brock, 2000). DCVC is the most potent of the TCE metabolites as a *Salmonella* mutagen. DCVG is not as potent as DCVC, but this is consistent with the hypothesis that DCVG needs to be converted to DCVC to induce point mutations. Although DCVC and DCVG have not been evaluated in other screening assays, there is some indication that they can induce primary DNA damage in mammalian cells in vitro and in vivo. Further, formation of covalent adducts with proteins and other macromolecules in mitochondria have been documented (Lash et al., 2000b). The covalent binding of DCVC is largely dependent on its metabolism by the $-lyase pathway. DCVC has been shown to induce the express of two proto-oncogenes, *c-fos* and *c-myc* (Lash et al., 2000b). At noted in the liver mode-of-action discussion, *c-myc* is involved in the control of cell proliferation and apoptosis (Tao et al., 2000a).

A key question in risk assessment, like that identified in the liver mode-of-action discussion, is whether TCE-induced kidney tumors are mediated through a compound- or metabolite-induced mutational mechanism. Two reports provide suggestive evidence for TCE-induced mutations in the VHL gene of renal cell carcinoma patients (Brüning et al., 1997b; Brauch et al., 1999). Brauch et al. (1999) expanded the work of Brüning et al. (1997b) and observed somatic mutations in 75% of the renal cell carcinomas from TCE-exposed individuals. Many (42%) of the carcinomas taken from TCE-exposed cases had multiple mutations, and nucleotide base changes were found in five regions. A specific "hot spot" on gene nucleotide 454 was altered in 13 renal cell carcinoma patients with TCE exposure, but this mutation was not detected in renal cell carcinoma patients without TCE exposure or was carried by healthy subjects. Brauch et al. (1999) hypothesize that the C>T mutational changes in the VHL gene of TCE-exposed renal cell carcinoma patients may be associated with alkylation and DNA adduct formation of the opposite DNA strand. It is probable that these mutations play a role in the development of these tumors because a function of this gene is to suppress the development of kidney cancer. These data identify a profile that seems to be specific to TCE exposure. The finding of the mutation in nucleotide 454 in normal kidney parenchymal tissue from four patients adds further support that this mutation may precede tumor formation. The halothioketenes, products from $-lyase cleavage of DCVC, form DNA adducts in vitro and could conceivably contribute to the induction of these mutations (Müller et al., 1994, 1998a,b). Moreover, chlorothioketenes have been shown in vitro to form adducts with cytosine in an aqueous solution (Volkel et al., 1998). There may, however, be alternative mechanisms such as selection of cells within the mutation, rather than producing the mutation, that may account for or at least contribute to these observations. Although this is not conclusive proof of a mutagenic mechanism, it is prudent to consider this as an important and likely mode of action and, at the minimum, a marker for TCE-induced kidney cancer.

3.5.2.2. *Accumulation of Alpha-2u Globulin*

In the past, the production of alpha-2u globulin was considered as contributing to TCE-induced kidney tumors, with the initiation of several studies to examine this hypothesis. Alpha-2u globulin accumulation is unique to male rats, and when in excess is associated with nephropathy, cytotoxicity, cellular proliferation, and consequent renal tumors. Goldsworthy shows that TCE does not cause an accumulation of alpha-2u globulin in the male (Goldsworthy et al., 1988; Lash et al., 2000b). Moreover, TCE is a renal toxicant and clearly causes kidney damage in both male and female rats and in mice (Barton and Clewell, 2000). With respect to kidney tumors, the analysis of Rhomberg (2000) shows that kidney tumors are found in both TCE-treated male and female rats. Thus, alpha-2u globulin accumulation does not appear to be a

mode of action of TCE-induced kidney toxicity in rats or relevant to human risk (Lash et al., 2000b).

3.5.2.3. *Peroxisome Proliferation*

A potential role of peroxisome proliferation should be included in any mode-of-action discussion for kidney tumor induction because renal enzymes generate chloroacetates that are known to induce peroxisome proliferation, and the renal proximal tubular epithelial cells are relatively rich in peroxisomes. One study specifically has addressed the issue of TCE-induced peroxisome proliferation in the kidney, with other studies examining the influence of TCE metabolites on peroxisome proliferation (Lash et al., 2000b). Peroxisome proliferation was reported to occur in both rat and mouse kidney following exposure to TCE, with mice showing a greater response, as measured by cyanide-insensitive palmitoyl-CoA oxidation, than that seen in rats (Goldsworthy and Popp, 1987). TCE and its metabolites have not been shown to induce kidney cancer in mice, however. Odum et al. (1988), additionally, suggest that TCA-induced peroxisome proliferation does not play a role in male rat kidney carcinogenicity. A role for peroxisome proliferation of TCE-induced kidney carcinogenicity is unlikely.

3.5.2.4. *Nephrotoxicity and Cytotoxicity*

TCE has been demonstrated to cause nephrotoxicity and to induce persistent changes to the proximal tubules in both rats and humans. It has been proposed that kidney tumor induction results from cellular necrosis and activation of repair processes that lead to cellular proliferation. Most of the studies aimed at elucidating this possible mode of action have focused on DCVG or DCVC, rather than the parent compound, as it is believed that flux through the GSH conjugation pathway and the subsequent production of reactive species are responsible for observed nephrotoxicity (Lash et al., 2000b). The in vivo formation in both animals and humans of these intermediates from TCE further adds significance to these studies in an assessment of TCE. In spite of the importance of $-lyase metabolism in cysteine conjugated-induced nephrotoxicity, there is evidence in several other studies that suggests additional enzymatic activities may also bioactivate DCVC and may be important to nephrotoxicity (Lash et al., 2000b).

A number of in vivo and in vitro studies support that TCE and its cysteine conjugates are associated with nephrotoxicity, specifically proximal tubular damage, as measured by elevated excretion of renal enzymes or by histopathologic examination. Nephrotoxicity has also been shown in individuals with occupational exposure to high levels of TCE (Lash et al., 2000b; Brüning et al., 1999a, 1999b). Nephrotoxicity as well as tumors were observed concurrently in the NTP bioassay (NTP, 1988). The incidence of nephrotoxicity in the rat bioassay was high, however, and far exceeded the reported incidence of tumors (NTP, 1988). Further,

nephrotoxicity appears to correlate with the known sex differences in metabolism rates, males having a higher metabolic rate than females (Lash et al., 2000b). Exposure to TCE was associated with a modest increase in lactate dehydrogenase release (a marker of cell injury) in male rat kidney cells, but not in cells from female rats at similar concentrations (Lash et al., 2000b).

Nephrotoxicity may result from oxidative stress, disturbances in calcium ion homeostasis, mitochondrial dysfunction, or protein alkylation. A number of studies demonstrate nephrotoxicity by these process with exposure to TCE or, more so, to DCVC. Both TCE and DCVC induce an oxidative stress response that includes GSH oxidation or depletion, lipid peroxidation, and oxidation or alkylation of protein sulfhydryl groups after exposure of renal cells to these agents. It is likely that oxidative stress plays some role in DCVC-induced nephrotoxicity, but lipid peroxidation is probably a consequence rather than a cause of cellular injury (Lash et al., 2000b). DCVC-induced changes in free calcium ion concentrations include inhibition of mitochondrial metabolism and function, severe mitochondrial damage, poly(ADP)-ribosylation of nuclear proteins and DNA double-strand breaks, and changes in cytoskeletal protein structure. The cysteine conjugates, including DCVC, have been shown to covalently bind with proteins and other macromolecules in mitochondria. Cytosolic proteins are another target of the reactive metabolites of DCVC, and protein adducts have been observed in vitro in human proximal tubular cells with exposure to DCVG and DCVC and in rats exposed in vivo to DCVC. The finding of higher levels of covalent binding in TCE-exposed mice compared to rats suggests that other factors besides covalent adduct formation must contribute to the induction of renal carcinogenesis (Lash et al., 2000b).

Recently, Green (Green et al., 1998) put forth another hypothesis that urinary excretion of formic acid leading to decreased pH after TCE exposure may partially explain the observed renal toxicity in the TCE studies. The excretion of increased levels of formic acid as observed with exposure to TCE appears to be related to folate deficiency (Dow and Green, 2000). Kidney toxicity has been reported in humans and rabbits with exposure to formic acid (Jacques, 1982; Liesivuori and Savolainen, 1986, 1987, 1991, 1992); however, data are lacking on whether formic acid induces kidney tumors. At the present time there is limited evidence that this pathway is involved with renal tumorigenesis. Moreover, demonstrating a role for this mechanism does not detract from a potential contribution to tumorigenesis by other pathways, e.g., glutathione conjugation and processing to DCVC and subsequent reactive metabolites.

3.5.2.5. *Contribution of Several Modes of Action*

It is likely that multiple modes of action may be important in TCE-induced kidney cancer (Lash et al., 2000b). As in the liver, level of exposure will be an important consideration. The

preponderance of the evidence suggests that the cysteine conjugates and reactive metabolites generated from their $-lyase metabolism are most likely responsible for kidney toxicity and tumorigenicity (Lash et al., 2000b). These metabolites have not been tested in a chronic bioassay and the lack of such information is an uncertainty associated with this inference. More information is also needed regarding any potential role of the oxidative metabolites of TCE and of formic acid.

Separating the contribution of genotoxicity from that of nephrotoxicity and chronic cellular injury in relation to the development of renal tumors is important to a discussion of potential modes of action for kidney cancer and TCE. The cysteine metabolites appear to be inextricably intertwined such that the contribution of these events in tumor development cannot be evaluated on the basis of data that are available today. As DCVC has been demonstrated to be mutagenic in *Salmonella*, and mutation in the VHL gene seems to be associated with TCE-related tumors in epidemiological studies, it is reasonable to regard gene mutation as a potential mode of action for kidney tumors.

3.5.3. Lung Cancer

There has been much less investigation into potential modes of action for TCE-induced lung cancer than for the liver and kidney. Green (2000) suggests that mice may be more sensitive to the toxic effects of TCE exposure in the lung than are other species tested because of their increased amounts of CYP2E1 metabolism, specifically in Clara cells, compared to humans and rats. Lung tumors in mice are thought to arise through an accumulation of CH in the Clara cells of the lung, causing cell damage and compensatory cell replication, which in turn leads to tumor formation (Green, 2000). CH is believed to be the etiologic agent because independent studies have shown that CH when administered alone causes lung lesions identical to TCE-induced tumors. Neither TCA nor TCOH (metabolites of CH) causes lung lesions.

This hypothesis suggests that such a mechanism in mice may not be extrapolated to humans, as there is little CYP2E1 activity in human lungs as a whole (Green, 2000). However, activity from whole lungs may give misleading results because of the variety of cell types. High activity for a few cell types may be diluted by other cells with low activity (Lash et al., 2000b). While thought to be relatively scarce in the human lung, recently Boers et al. (Boers et al., 1999) have characterized the number of Clara cells in the normal human airway and show that Clara cells contribute substantially to cell renewal in normal conducting airway epithelium in humans. Furthermore, Clara cells have been identified as important to the development of lung adenocarcinoma in humans (Boers et al., 1999).

The hypothesis that CH is responsible for lung tumor induction requires further confirmation. It would be useful to develop strong connections between metabolism of TCE, the

actual tumor cell of origin, and the contribution of other possible mechanisms that CH might utilize to increase lung tumor incidence. CH is clearly clastogenic and mutagenic at high doses (Moore and Harrington-Brock, 2000), raising the possibility of genotoxic activity as well as a mode of action involving simple cytotoxicity and reparative hyperplasia. Moreover, it is not yet clearly demonstrated that CH is the metabolite responsible for lung tumor development.

3.5.4. Toxicity in Other Organs

Mode-of-action information for toxicity in other organs such as the endocrine and lymphopoietic system, and in the developing fetus, is more limited than that for the liver, kidney and lung. Data specific to TCE and its metabolites are few, and support for mode-of-action hypotheses are based on observations of common activities with other agents. In some cases, the roles of PPAR activation or cell signaling perturbations that are relevant to mode-of-action discussions for the liver and kidney are applicable to discussions of toxicity observed in these other organ systems. In other cases, such as lymphoma seen in the human epidemiologic studies, studies using animal models are lacking or are too few to support the human observations.

Endocrine system effects associated with TCE exposure include the development of testicular (Leydig cell) cancer in rats (Maltoni et al., 1988; NTP, 1988) and hormone disturbances (SHBG, DHEAS, testosterone) in humans (Chia et al., 1997; Goh et al., 1998). TCE and its metabolites, TCA and TCOH, have been found to partition in the male reproductive organs of rats after 6 weeks of inhalation exposure (Zenick et al., 1984). A wide variety of agents that affect steroid hormone levels, such as testosterone, estradiol, and luteinizing hormone, will also induce Leydig cell tumors in the rat (Cook et al., 1999). Peroxisome proliferating chemicals have been shown to induce Leydig cell tumors via a modulation of growth factor expression by estradiol (Cook et al., 1999). Peroxisome proliferating chemicals induce hepatic aromatase activity, which can increase serum and testis estradiol levels. The increased interstitial fluid estradiol levels can modulate growth factors, including TGF", and stimulate Leydig cell proliferation (Cook et al., 1999).

Steroid hormones such as testosterone, estradiol, and luteinizing hormone are regulated through the hypothalo-pituitary-testis (HPT) axis in both rats and humans, and agents that induce Leydig cell tumors in rats by disruption of the HPT axis are thought to pose a hazard to humans (Cook et al., 1999). Cook et al. (1999) further suggest that Leydig cell tumors induced through an aromatase activity mode-of-action such as that suggested for peroxisome proliferators are considered potentially relevant to humans. Although the risk of Leydig cell cancer in humans may be diminished compared to rats owing to the absence of SHBG, the observation of Leydig cell tumors in rats exposed to TCE may act as a signal for disturbance of the endocrine system and be indicative of potential endocrine disturbances in humans. Further, a potential target of

testosterone disruption may be the prostate, which in humans is sensitive to testosterone and other androgens (Shimada et al., 1999; Bosland et al., 1995) and is affected by Leydig cell function in the rat (Yount et al., 1982). The effects of endocrine disruption in human populations exposed to TCE are an area for more research.

TCE exposure has been associated with effects on immune system functioning and includes both immune suppression and autoimmunity. Reports of both immune suppression and autoimmunity may appear paradoxical at first glance; however, these observations are plausible. Certain elements of the immune system may be differentially affected by a chemical, affecting the ability to mount well-regulated immune responses to both foreign and self-antigens and leading to conditions such as autoimmunity via an alteration in the balance between specific immune components. Immune suppression, furthermore, may play a role in the induction of cancer; many immune-suppressive agents are human carcinogens (Tomatis et al., 1989). Additionally, a common event or events may exist in mode-of-action pathways for tumorigenesis and for autoimmunity (e.g., dysregulation of apoptosis). The observation of an increased risk of multiple myeloma in patients with autoimmune diseases provides some support for this hypothesis (Cooper et al., 1999).

Organic solvent exposure in general is associated with autoimmune disease such as scleroderma (systemic sclerosis) (Nietert et al., 1998). Mode-of-action hypotheses for autoimmunity resulting from environmental agents have been grouped into three broad categories: (1) a change in the hormonal milieu to favor estrogenic stimulation of the immune system; (2) suppression of one section of the immune system, which disrupts normal immune surveillance; and (3) chemical binding to a self-antigen forming a neoantigen, thus breaking tolerance by inducing immunity to the unmodified native molecule as well as to the modified antigen (Mayes, 1999). These mechanisms are currently speculative at best, but are supported by animal models (Mayes, 1999). TCE exposure, specifically, has been shown to induce autoantibody formation against dichloroacetyl chloride in genetically susceptible autoimmune-prone mice (Griffin et al., 2000a,b, Khan et al., 1995). Moreover, T-cells were shown to secrete more interferon-(and less interleukin-4 after TCE exposure (Griffin et al., 2000a). Griffin et al., (2000a) note that the pattern of response was consistent with a T-helper Type 1 immune or inflammatory response. Furthermore, these effects have been shown to contribute to the development of autoimmune disease in the liver (autoimmune hepatitis) (Griffin et al., 2000c). Oxidative metabolism is necessary for inducing the inflammatory response because blocking the CYP450 pathway was shown to primarily inhibit the response (Griffin et al., 2000b). One epidemiologic study also reported an strong association between TCE exposure and systemic sclerosis patients with autoantibodies (Nietert et al., 1998). The mode(s) of action for these observations is not known at this time.

The Kupffer cell may also play an important role in autoimmune response. Kupffer cells have been discussed previously in the liver mode-of-action section. Activated Kupffer cells release a number of chemicals, including hydrogen peroxide, superoxide ion, and hydrolytic enzymes, which aid in antigen destruction, and also cytokines, which have immunoregulatory and inflammatory activity (e.g., interleukins, TGF", and TGF$). Several of these chemicals have also been associated with apoptosis (see liver cancer discussion) (Laskin, 1996). Treatment of mice with TCE for 30 days has been shown to affect Kupffer cells and the production of inflammatory cells (Kjellstrand et al., 1983). Moreover, peroxisome-proliferating chemicals in general are also reported to affect Kupffer cell function (Youseff and Badr, 1998), and PPAR activation may play a role in autoimmune disease.

Immune suppression and TCE exposure are areas needing more research. Immune suppression may play a significant role in the development of non-Hodgkin's lymphoma, as seen in the epidemiologic studies (Wartenberg et al., 2000) and in the mouse bioassay (Henschler et al., 1980). Non-Hodgkin's lymphoma is found with a higher frequency among individuals with compromised immune systems resulting from either viruses or past chemotherapy (Smith, 1996). Although this is not well studied, one report of TCE exposure to female mice via the drinking water shows exposure-related inhibition of humoral immunity with effects on bone marrow function, cell-mediated immune responses, and macrophage function (Sanders et al., 1982).

Mode-of-action hypotheses for observed developmental effects seen with TCE, TCA, and DCA exposure are not well developed, and data specific to TCE exposure are few. Developmental effects that have been associated with TCE or TCE metabolite exposure include micropthalmia/anopthalmia (eye anomalies) in rats, cardiac defects in rats and humans and, more vaguely, neural tube defects in humans. Micropthalmia has been reported in human offspring with maternal alcohol and retinoic acid exposures. Both retinoic acid and ethanol have in common peroxisome receptor activity. PPAR" activation may be important to the development of eye anomalies, although no data currently support this hypothesis. In fact, another agent that activates PPAR, DEHP, induces pronounced anopthalmia and micropthalmia in rats (Narotsky et al., 1995a,b).

Researchers at the University of Arizona are examining the cardiac teratogenicity of trichloroethylene. Research is focused on evaluating whether gene expression is affected during cardiogenesis by altering several molecules which are hypothesized to be critical for normal heart development. Recently Boyer et al. (2000) have reported that TCE treatment produces a dose-dependent inhibition of mesenchymal cell transformation (a critical event in development of the heart) in progenitors of the valves and septa in the heart in vitro. Boyer et al. (2000) also note a concurrent study that shows alteration of gene expression in rat embryo hearts with maternal exposure in drinking water of 110 ppm TCE. Although questions may surround the experimental

evidence of whether cardiac anomalies observed in the developmental assays are related to TCE, Boyer et al. (2000) report that TCE can affect events important to the development of the heart, events which are consistent with an induction of cardiac anomalies. Moreover, the TCE metabolites, TCA and DCA, both produce cardiac anomalies in rats (Smith et al., 1989, 1992; Johnson et al., 1998a,b; Epstein et al., 1993). DCA also concentrates in rat myocardial mitochondria (Kerbey et al., 1976), freely crosses the placenta (Smith et al., 1992), and has known toxicity to tissues dependent on glycolysis as an energy source (e.g., the testes, lens, and nervous system in humans and dogs) (Yount et al., 1982; Stacpoole et al., 1979; Katz et al., 1981; Cicmanec et al., 1991). Mammalian embryos also rely on glycolysis for energy, therefore suggesting a common vulnerability for toxicity (Smith et al., 1992). However, there is no direct evidence for DCA to affect glycolysis in the embryo. More research into TCE and its metabolites is needed to more fully elucidate possible modes of action for the effects observed in standard developmental protocols.

3.6. HAZARD CHARACTERIZATION

3.6.1. Consideration of Causality in the Epidemiologic Evidence

Overall, the epidemiologic studies suggest an association between TCE exposure and excess risks of kidney cancer, liver cancer, lympho-hematopoietic cancer, cervical cancer, and prostate cancer (Wartenberg et al., 2000). Determining whether an association is one of cause and effect involves considering several aspects that are characteristic of cause-and-effect relationships (Hill, 1965; Rothman, 1986).[45] No aspect is either necessary or sufficient; rather, they must be weighed to determine whether the most likely explanation of an observed association is cause and effect.

(1) Strength of the observed association. The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. A modest risk, however, does not preclude a causal association and may reflect a lower level of exposure or an agent of lower potency. For TCE, the observed risks are generally modest (twofold or less) for cancers of the kidney, liver, and lympho-hematopoietic system. An exception is the large odds ratio for kidney cancer (OR=10.8, 95% CI= 3.4-34.8) reported by Vamvakas et al. (1998). Differences between cohort and case-

[45]EPA's proposed cancer guidelines (U.S. EPA, 1999) use this framework to determine whether a cause-and-effect interpretation of the epidemiologic evidence is credible. The proposed guidelines may extend this framework to the separate question of determining whether the mode-of-action information is sufficient to establish cause and effect. For this question, the key aspect of causality is whether experimental evidence in the laboratory demonstrates that a specific sequence of key events leads to the observed tumors and that intervening to prevent a key event will prevent tumor formation.

control studies, in part, may reflect exposure differences. The observed risks are not thought to be attributable to smoking (important in liver cancer) or socioeconomic status (important in cervical cancer).

(2) Consistency of the observed association. An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. Some degree of consistency was observed for associations of TCE exposure with cancers of the kidney, liver, lympho-hematopoietic system, cervix, and prostate (Wartenberg et al., 2000). Consistency is strongest for kidney cancer, supported by tier-I, tier-II, and tier-III mortality (but not tier-III incidence) studies and case-control studies (see Section 3.4.2). For liver cancer, consistent results were observed in tier-I and tier-II but not tier-III studies (see Section 3.4.1). For lympho-hematopoietic cancer, associations observed in the tier-I studies were supported by the ecologic studies of residential drinking water exposure, though not by the tier-II or tier-III studies (see Section 3.4.4). Cervical cancer, though sparsely reported, is elevated in tier-I incidence studies and tier-III mortality studies (see Section 3.4.5). Prostate cancer is elevated in the tier-I studies (see Section 3.4.5).

(3) Specificity of the observed association. Traditionally, specificity has been defined in terms of one cause, one disease (Hill, 1965). TCE causes cancer at several sites in rats and mice; hence, there is no expectation that TCE would be associated with only one human cancer. Many agents cause cancer at multiple sites, and many cancers have multiple causes. Evidence of specificity may come from the recent observation of multiple mutations of the VHL tumor suppressor gene, primarily C>T changes including nucleotide 454, in renal cell carcinoma patients with high, prolonged TCE exposure (Brauch et al., 1999; Brüning et al., 1997b) (see Section 3.5.2). Key research related to specificity would include investigation of VHL gene mutations in other cohorts exposed to TCE (see Section 5).

(4) Temporal relationship of the observed association. A causal interpretation is strengthened when exposure is known to precede development of the disease. Associations between TCE exposure and several forms of cancer are supported primarily by cohort and case-control studies, in which the temporal relationship is well described. All drinking water studies, except the case-control analysis by MA-DOH (1997), are ecologic or prevalence studies, in which knowledge of the temporal relationship is lacking. For this reason, the conclusions place greater weight on the cohort and case-control studies.

(5) Biologic gradient (exposure-response relationship). A clear exposure-response relationship strongly suggests cause and effect. For TCE, biologic gradients are only sporadically observed, though the number of site-specific cancers in a study is often too small to identify biologic gradients. For kidney cancer, only the studies by Morgan et al. (1998) and Vamvakas et al. (1998) observed exposure-response relationships.

Both the urinary biomarker studies and the Blair et al. (1998) study observed liver cancer incidence to increase with exposure. Anttila et al. (1995) observed incidence to increase with time since first exposure, with the largest risk among those with the longest time since first exposure (SIR=6.1, 95% CI= 2.8-17.7). Similar findings were not observed for liver cancer mortality; however, liver cancer incidence is considered more definitive because liver cancer mortality is extensively miscoded on death certificates.

Exposure-response analyses presented in the drinking water studies collectively suggest that greater exposure to drinking water contaminated with TCE and other chlorinated solvents is associated with lymphatic cancer, particularly leukemia. Studies by Cohn et al. (1994), Fagliano et al. (1990), and MA-DOH (1997) reported exposure-response relationships between drinking water exposure to TCE and other chlorinated solvents and the risk of lympho-hematopoietic cancer, particularly childhood leukemia and non-Hodgkin's lymphoma.

For cervical cancer, Anttila et al. (1995) and Blair et al. (1998) observed incidence to increase with exposure, with a significantly elevated risk among those with the highest urinary TCA levels in the Anttila study (SIR=4.4, 95% CI= 1.4-10.1). Comparison with internal controls in the Blair study suggests that TCE may be the etiologic agent rather than a confounder such as socioeconomic status.

(6) Biologic plausibility. Section 3.5 discusses the many recent mechanistic studies investigating TCE's carcinogenic effects in rats and mice and their relevance to humans. The mechanistic results to date indicate that TCE-induced carcinogenesis is complex, involving multiple carcinogenic metabolites acting through multiple mechanisms, all of which have relevance to humans (see Section 3.5).

(7) Coherence. A cause-and-effect interpretation of the human data does not conflict with other lines of evidence. The strongest associations between TCE exposure and human cancer are for the kidney, liver, and lympho-hematopoietic system, sites where TCE causes cancer in either rats or mice. In both humans and laboratory animals, TCE can cause adverse effects other than cancer in the kidney, liver, and immune system, and it is biologically plausible that some of these effects may play a role in the development of cancer of the kidney, liver, and lympho-hematopoietic system, respectively (see Sections 3.5.2, 3.5.1, 3.5.4). The associations between human cervical and prostate cancer have no suitable animal models to compare.

(8) Experimental evidence (from human populations). Experimental evidence is seldom available from human populations (Rothman, 1986) and exists only when conditions of human exposure are altered to create a "natural experiment" at different levels of exposure. For example, leukemia cases became more evenly distributed throughout the town of Woburn after closure of two wells contaminated with TCE and other solvents (MA-DOH, 1997).

(9) Analogy. The pattern of effects associated with TCE, particularly in the liver, has similarities to those of several other chlorinated solvents and to mixed-solvent exposures.

3.6.1.1. *Synthesis of the Epidemiologic Evidence*

Together, these nine aspects of causality suggest that a cause-and-effect association between TCE exposure and cancer is credible. Among the different tumor sites, support is strongest for kidney cancer, followed by liver and lympho-hematopoietic cancer and then cervical and prostate cancer.

The strength of this conclusion is tempered by some still-open questions about TCE's cancer potential. The statistical significance of the results of the joint epidemiologic analysis could change with omission of one study or another. Most studies lack quantitative exposure information. More research is needed on mutations of the VHL gene, particularly in renal cell carcinoma patients not exposed to TCE. In addition, a nested case-control study could help resolve questions about causality, as this study design has the potential to examine the effects of lifestyle factors and exposures to mixtures of solvents that may share common metabolites and modes of action (see Section 5).

3.6.2. Weight of Evidence Under the Current and Proposed Cancer Guidelines

TCE has been extensively tested in animals, with mice developing liver tumors, lung tumors, and lymphomas, and rats developing kidney tumors and testicular tumors. Epidemiologic studies, considered as a whole, have associated TCE exposure with excess risks of kidney cancer, liver cancer, lympho-hematopoietic cancer, cervical cancer, and prostate cancer. Weight-of-evidence characterizations have had to address issues concerning the strength of the human evidence and the relevance of the animal tumors to humans.

With this assessment, the weight of the epidemiologic evidence of TCE's potential carcinogenicity has become stronger than before (see Section 3.4). To bridge the opposing views of the past, this assessment commissioned a joint analysis of the epidemiologic studies (Wartenberg et al., 2000). This joint analysis used a statistically based weight-of-evidence approach that stratified the available studies into tiers according to how well each study's results

can be associated with TCE exposure specifically.[46] Within each tier, study results were weighted according to inverse variance.[47] The joint analysis found that the most consistent and compelling results are statistically significant increased incidences of kidney cancer and liver cancer in workers exposed to TCE. These are followed by lympho-hematopoietic cancer, cervical cancer, and prostate cancer. There are few studies of highly exposed populations, consequently, the magnitude of response is generally less than a twofold increase. The joint analysis leads to a conclusion that these increases are statistically significant, thus, TCE may be capable of causing cancer in humans at multiple sites.

The epidemiologic studies have recently been augmented by molecular information, in which multiple mutations of the VHL tumor suppressor gene,[48] primarily C>T changes including nucleotide 454, were found in renal cell carcinoma patients with high, prolonged TCE exposure (Brüning et al., 1997b) (see Section 3.5.2). A followup study found mutations of the VHL gene in 75% of TCE-exposed workers with renal cell carcinoma, with an association between number of mutations and TCE exposure level (Brauch et al., 1999). Adding specificity to this association, a C>T change at nucleotide 454 was found in 13 of 33 renal cell carcinoma patients with TCE exposure; this specific mutation was not found in those without identifiable TCE exposure.

The mechanistic research into the mode-of-action for each animal tumor site has begun to link TCE with disturbances in cell signaling and carbohydrate metabolism, which can lead to human cancer and other diseases (see Section 3.5). Subject to dose-response adjustments for relative human-to-animal sensitivity (see Sections 4.5.2, 4.5.3), this research makes it plausible that TCE acts through mechanisms that can cause cancer in humans.

EPA's proposed cancer guidelines provide that molecular information can be used in choosing a weight-of-evidence descriptor. TCE could be described as "carcinogenic to humans," supported by (1) association of TCE exposure with increased risk of human kidney cancer, liver

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[46]Tier I studies are those in which TCE exposure has been inferred for individual study subjects and in which it is best characterized. Tier II studies are those in which there is putative TCE exposure, but individuals are not identified as uniquely exposed to TCE. Tier III studies are the studies of dry cleaning and laundry workers in which subjects are exposed to a variety of solvents including TCE (Wartenberg et al., 2000).

[47]*Inverse variance* gives greater weight to larger studies that yield more precise estimates. Study results are averaged, with each study result weighted (i.e., multiplied) by a factor that is inversely proportional to the study result's variance.

[48]The VHL gene normally suppresses renal cell carcinomas. Mutations to this gene have been noted in kidney cancers and may be an important risk factor and mode-of-action for chemically induced renal cell cancer (Lash et al., 2000b). To illustrate the difference between "mode" of action and "mechanism" of action, knowledge that VHL gene mutations are involved may be enough to identify a plausible "mode" of action, whereas knowing how such modifications induce subsequent events leading to kidney cancer would be needed to identify the "mechanism" of action.

cancer, lympho-hematopoietic cancer, cervical cancer, and prostate cancer; (2) induction of kidney tumors and testicular tumors in rats and liver tumors, lung tumors, and lymphomas in mice; and (3) induction of multiple VHL gene mutations, primarily C>T changes including nucleotide 454, in a human population. At this time, however, fuller identification of the mode-of-action and associated key events is lacking (see Section 5). It is not known whether the C>T change at nucleotide 454 is a marker of exposure, a key event leading to kidney cancer, or whether other factors selectively favor the growth of cells with this change. Functional alteration of the VHL gene can be a mode of kidney cancer induction; whether this is how TCE induces kidney cancer has not been established.

Alternatively, TCE could be described as "likely to be carcinogenic to humans," supported by evidence associating TCE exposure with human cancer and strong evidence of carcinogenicity in animals involving processes that have relevance for humans. Among "likely" carcinogens, the evidence for TCE is strong and is drawn from several types of information (see Sections 3.2–3.5).

At this time, a strong characterization as "highly likely to be carcinogenic to humans" is more appropriate, given the still-open questions about TCE's cancer potential. The statistical significance of the results of the joint epidemiologic analysis could change with omission of one study or another. This does not decrease confidence in the results of the joint analysis, but emphasizes that the TCE studies collectively have only recently accumulated enough power to begin to detect associations between moderate TCE exposures and some relatively common cancers. On the other hand, the new evidence associating TCE exposure with a transformation at nucleotide 454 is striking evidence specific to TCE exposure, putting a "genetic fingerprint" that associates such kidney tumors with TCE exposure. Replication of this result in another cohort showing kidney tumors could warrant a future description of TCE as "carcinogenic to humans" (see Section 5).

Under the current (1986) cancer guidelines, TCE would be classified as a "probable human carcinogen" (group B1), with "limited" human evidence and "sufficient" animal evidence of carcinogenicity. The principal changes from the controversy of the late 1980s are the stronger weight of epidemiologic evidence (see Section 3.4) and the new mechanistic information suggesting that TCE's modes of action may be relevant to humans (see Section 3.5).

Table 3-1. Liver tumors in mice exposed to TCE

Hepatocellular carcinomas and adenomas after 103 weeks gavage exposure, beginning at 8 weeks of age (NTP, 1990)

Administered dose (mg/kg-d)	Vehicle ctl	1,000
Male B6C3F1 mice **	14/48 (29%)	39/50 (78%)
Female B6C3F1 mice **	6/48 (13%)	22/49 (45%)

Hepatocellular carcinomas[a] after 90 weeks gavage exposure, beginning at 5 weeks of age (NCI, 1976)

Dose group	Vehicle ctl	Low dose[b]	High dose[c]
Male B6C3F1 mice **	1/20 (5%)	26/50 (52%)	31/48 (65%)
Female B6C3F1 mice **	0/20 (0%)	4/50 (8%)	11/47 (23%)

Hepatomas after 78 weeks inhalation exposure, beginning at 11-12 weeks of age (Maltoni et al., 1986)

Administered daily concentration (mg/m^3)[d]	Control	112.5	337.5	675
Male Swiss mice[e] **	4/66 (6%)	2/53 (4%)	8/59 (14%)	13/61 (21%)
Female Swiss mice[e]	0/84 (0%)	0/89 (0%)	0/86 (0%)	1/86 (1%)
Male B6C3F1 mice[f] **	1/59 (2%)	1/31 (3%)	3/38 (8%)	6/37 (16%)
Female B6C3F1 mice[f] **	3/88 (3%)	4/89 (4%)	4/88 (5%)	9/85 (11%)
Male B6C3F1 mice[g]	17/77 (22%)	19/47 (40%)	27/67 (40%)	21/63 (33%)

**Statistically significant by Cochran-Armitage trend test ($p<0.05$).
[a]Hepatocellular adenomas were not reported.
[b]Low dose is 1,200 mg/kg-d for male mice, 900 mg/kg-d for female mice (5 d/wk).
[c]High dose is 2,400 mg/kg-d for male mice, 1,800 mg/kg-d for female mice (5 d/wk).
[d]Equivalent to 100, 300, 600 ppm (100 ppm=540 mg/m^3), adjusted for 7 hr/d, 5 d/wk exposure.
[e]Mice alive at week 43, when first hepatoma was observed.
[f]Mice alive at week 33, when first hepatoma was observed (expt BT306).
[g]Mice alive at week 68, when first hepatoma was observed (expt BT306 bis).
Sources: NTP *(1990)* tables 8, 9; NCI *(1976)* table VIII; Maltoni et al *(1986)* IV/VI table 14, IV/VII table 14, IV/VIII table 14.

Table 3-2. Kidney tumors in rats, adjusted for reduced survival[a]

Tubular cell adenomas and adenocarcinomas after 103 weeks gavage exposure, beginning at 6.5-8 weeks of age (NTP, 1988, 1990)

Administered dose (mg/kg-d)	Untreated ctl	Vehicle ctl	500	1,000
Male ACI rats	0/48 (0%)	0/46 (0%)	1/29 (3%)	0/22 (0%)
Male August rats	0/47 (0%)	0/47 (0%)	2/35 (6%)	1/33 (3%)
Male Marshall rats	2/43 (4%)	0/44 (0%)	1/25 (4%)	1/28 (4%)
Male Osborne-Mendel rats **	0/46 (0%)	0/47 (0%)	6/44 (14%)	2/33 (6%)
Male F344/N rats **	0/48 (0%)	0/46 (0%)	2/46 (4%)	3/33 (9%)
Pooled males **	2/232 (1%)	0/230 (0%)	12/179 (7%)	7/149 (5%)
Female ACI rats	0/46 (0%)	0/43 (0%)	3/36 (6%)	1/31 (3%)
Female August rats	0/47 (0%)	1/47 (2%)	4/42 (10%)	0/34 (0%)
Female Marshall rats	1/48 (2%)	1/49 (2%)	2/44 (5%)	1/32 (3%)
Female Osborne-Mendel rats	1/47 (2%)	0/43 (0%)	0/44 (0%)	1/45 (2%)
Female F344/N rats	0/46 (0%)	0/46 (0%)	0/45 (0%)	1/44 (2%)
Pooled females **	2/234 (1%)	2/228 (1%)	9/211 (4%)	4/186 (2%)

Renal tubuli adenocarcinomas after 104 weeks inhalation exposure, beginning at 12 weeks of age (Maltoni et al., 1986)

Administered daily concentration (mg/m^3)[b]	Control	112.5	337.5	675
M Sprague-Dawley rats **	0/120 (0%)	0/118 (0%)	0/116 (0%)	4/122 (3%)
F Sprague-Dawley rats	0/139 (0%)	0/128 (0%)	0/127 (0%)	1/127 (1%)

**Statistically significant by Cochran-Armitage trend test ($p<0.05$).
[a]ACI, August, Marshall, Osborne-Mendel, and F344/N rats alive at week 57, Sprague-Dawley rats at week 47 (expt BT304) or week 62 (expt BT304 bis).
[b]Equivalent to 100, 300, 600 ppm (100 ppm=540 mg/m^3), adjusted for 7 hr/d, 5 d/wk exposure.
Sources: NTP (1988) Tables A2, C2, E2, G2; NTP (1990) table A3; Maltoni et al. (1986) IV/IV Table 19, IV/V Table 19.

Table 3-3. Malignant lymphomas in mice exposed to TCE

Malignant lymphomas after 103 weeks gavage exposure, beginning at 8 weeks of age (NTP, 1990)

Administered dose (mg/kg-d)	Vehicle ctl	1,000
Male B6C3F1 mice	11/50 (22%)	13/50 (26%)
Female B6C3F1 mice	7/48 (15%)	13/49 (27%)

Lymphosarcomas and reticulum cell sarcomas after 90 weeks gavage exposure, beginning at 5 weeks of age (NCI, 1976)

Dose group	Vehicle ctl	Low dose[a]	High dose[b]
Male B6C3F1 mice	1/20 (5%)	4/50 (8%)	2/48 (4%)
Female B6C3F1 mice	1/20 (5%)	5/50 (10%)	5/47 (11%)

Malignant lymphomas after 78 weeks inhalation exposure (Henschler et al., 1980)

Administered daily concentration (mg/m^3)[c]	Control	96	480
Male Han:NMRI mice	7/30 (23%)	7/29 (24%)	6/30 (20%)
Female Han:NMRI mice **	9/29 (31%)	17/30 (57%)	18/28 (64%)

**Statistically significant by Cochran-Armitage trend test (p<0.05).
[a]Low dose is 1,200 mg/kg-d for male mice, 900 mg/kg-d for female mice (5 d/wk).
[b]High dose is 2,400 mg/kg-d for male mice, 1800 mg/kg-d for female mice (5 d/wk).
[c]Equivalent to 100 and 500 ppm (100 ppm=540 mg/m^3), adjusted for 6 hr/d, 5 d/wk exposure.
Sources: NTP *(1990)* tables 8, 9; NCI *(1976)* table XXXa; Henschler et al *(1980)* table 3a.

Table 3-4. Testicular tumors in male rats exposed to TCE, adjusted for reduced survival[a]

Interstitial cell tumors after 103 weeks gavage exposure, beginning at 6.5-8 weeks of age (NTP, 1988, 1990)

Administered dose (mg/kg-d)	Untreated ctl	Vehicle ctl	500	1,000
Male ACI rats	38/45 (84%)	36/44 (82%)	23/26 (88%)	17/19 (89%)
Male August rats	36/46 (78%)	34/46 (74%)	30/34 (88%)	26/30 (87%)
Male Marshall rats **	16/46 (35%)	17/46 (37%)	21/33 (64%)	32/39 (82%)
Male Osborne-Mendel rats	1/30 (3%)	0/28 (0%)	0/25 (0%)	1/19 (5%)
Male F344/N rats	44/47 (94%)	47/48 (98%)	47/48 (98%)	32/44 (73%)

Leydig cell tumors after 104 weeks inhalation exposure, beginning at 12 weeks of age (Maltoni et al., 1986)

Administered daily concentration (mg/m^3)[b]	Control	112.5	337.5	675
M Sprague-Dawley rats **	6/114 (5%)	16/105 (15%)	30/107 (28%)	31/113 (27%)

**Statistically significant by Cochran-Armitage trend test ($p<0.05$).

[a]ACI rats alive at week 70, August rats at week 65, Marshall rats at week 32, Osborne-Mendel rats at week 97, F344/N rats at week 32, Sprague-Dawley rats at week 81 (expt BT304) or week 62 (expt BT304 bis).

[b]Equivalent to 100, 300, 600 ppm (100 ppm=540 mg/m^3), adjusted for 7 hr/d, 5 d/wk exposure.

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

Table 3-5. Lung tumors in mice exposed to TCE

Pulmonary adenomas and adenocarcinomas after 78 weeks inhalation exposure, beginning at 11-12 weeks of age (Maltoni et al., 1986)

Administered daily concentration (mg/m^3)	Control	112.5	337.5	675
Male Swiss mice **	10/90 (11%)	11/90 (12%)	23/90 (26%)	27/90 (30%)
Female Swiss mice	15/90 (17%)	15/90 (17%)	13/90 (14%)	20/90 (22%)
Male B6C3F1 mice	2/90 (2%)	2/90 (2%)	3/90 (3%)	1/90 (1%)
Female B6C3F1 mice **	4/90 (4%)	6/90 (7%)	7/90 (8%)	15/90 (17%)

Pulmonary adenomas and adenocarcinomas by inhalation (Fukuda et al., 1983)

Administered daily concentration (mg/m^3)	Control	56	168	504
Female ICR mice **	6/49 (12%)	5/50 (10%)	13/50 (26%)	11/46 (24%)

Carcinomas, adenocarcinomas, and adenomas of the lung or alveoli after 90 weeks gavage exposure, beginning at 5 weeks of age (NCI, 1976)

Dose group	Vehicle ctl	Low dose[a]	High dose[b]
Male B6C3F1 mice	0/20 (0%)	5/50 (10%)	2/48 (4%)
Female B6C3F1 mice	1/20 (5%)	4/50 (8%)	7/47 (15%)

**Statistically significant by Cochran-Armitage trend test ($p<0.05$).
[a]Low dose is 1,200 mg/kg-d for male mice, 900 mg/kg-d for female mice (5 d/wk).
[b]High dose is 2,400 mg/kg-d for male mice, 1,800 mg/kg-d for female mice (5 d/wk).
Sources: Maltoni et al., 1986, Tables 48, 51; Fukuda et al. (1983); NCI (1976) Table XXV.

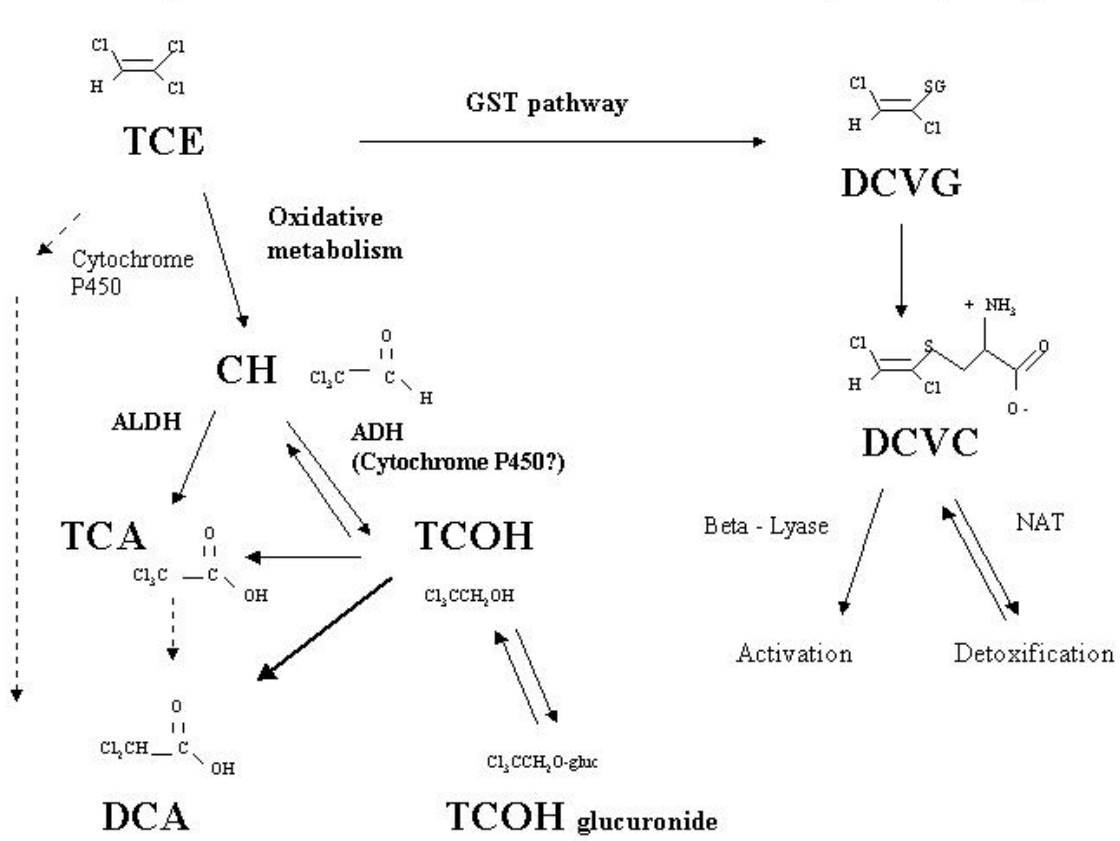

Figure 3-1. Metabolism of Trichloroethylene (TCE).

4. DOSE-RESPONSE ASSESSMENT AND CHARACTERIZATION

4.1. GENERAL CONSIDERATIONS AND APPROACH

This dose-response assessment updates the cancer slope factor for TCE and develops an oral RfD and an inhalation RfC for effects other than cancer. These effects include neurotoxicity, immunotoxicity, developmental toxicity, liver toxicity, kidney toxicity, endocrine effects, and several forms of cancer (see Section 3.4). The overall approach is to develop alternative estimates using different studies and modeling approaches, then to choose values that are supported by several sources of data and lines of reasoning. This provides a measure of confidence that the risk estimates are robust and not likely to be substantially changed by a new study or analysis.

This dose-response assessment draws from the state-of-the-science papers that appear in volume 1. Pharmacokinetic models[49] were developed to provide dose metrics[50] for the RfD, RfC, and cancer assessments (Fisher, 2000; Clewell et al., 2000). Statistical analyses improved these models by calibrating them to fit more data sets, then quantifying the uncertainty in each dose metric (Bois, 2000a,b). Analyses supporting the RfD and RfC applied pharmacokinetic models and empirical dose-response models[51] whenever suitable data were available (Barton and Clewell, 2000). The cancer assessment pursued several lines of analysis and considered both linear and nonlinear approaches, based on knowledge about mode-of-action at each tumor site (see Section 3.5). Empirical dose-response models were fitted to data on liver tumors and lung tumors in mice and kidney tumors and testicular tumors in rats (Rhomberg, 2000). A mechanism-based dose-response model[52] was developed for testing hypotheses about the mouse liver tumor response (Chen, 2000). Slope factors for kidney tumors, liver tumors, and non-Hodgkin's lymphoma were also developed from the epidemiologic data.

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[49]*Pharmacokinetic models* simulate the relationship between external exposure levels and the biologically effective dose at a target tissue. Pharmacokinetic models take into account absorption, distribution, metabolism, and elimination of the administered chemical and its metabolites. *Pharmacodynamic models* simulate the relationship between a biologically effective dose and the occurrence of a disease response.

[50]*Dose metrics* are alternative ways of describing dose. They involve what is being measured (parent compound, metabolite, or biomarker), where it is measured (whole body, blood, or specific tissue), and how it is measured (cumulative, average, or peak exposure). For example, total administered TCE and average TCA in liver are dose metrics.

[51]An *empirical dose-response model* is one based on fitting a curve to data. (They are sometimes called "curve-fitting" models or "benchmark dose" models.) In contrast to *mechanism-based dose-response models*, an empirical model is not based on specific knowledge about the biological mechanisms leading to disease.

[52]A *mechanism-based dose-response model* (also called a *biologically based dose-response model*) is a pharmacodynamic model whose mathematical structure reflects the ascertained mode-of-action and whose parameters are measured experimentally.

The assessment for effects other than cancer follows EPA's methods for developing RfDs (Barnes and Dourson, 1988) and RfCs (U.S. EPA, 1994). Despite the existence of numerous studies, however, the database for developing an RfD or RfC is problematic. ATSDR did not derive analogous chronic-duration levels[53] for TCE, viewing the chronic studies as limited by inadequate characterization of exposure, inadequate quantification of results, or lack of endpoints suitable for deriving chronic levels (ATSDR, 1997). This assessment, through modeling and comparing results for different adverse effects, species, exposure durations, and exposure routes, has overcome some data gaps and developed an RfD and RfC that are supported by multiple lines of reasoning.

The cancer assessment is consistent with the approach of EPA's proposed cancer guidelines (U.S. EPA, 1996). These guidelines encourage use of models to incorporate a wider range of experimental data and use of mode-of-action information to guide modeling and extrapolation approaches. This assessment develops alternative risk values from many sources before comparing and reconciling them. Because information about sensitive populations, children, and cumulative risks indicates the potential for vast human variation in risk, a range of cancer estimates is described, with guidance on how to choose a particular estimate based on risk factors in the exposed population and exposure scenario.

4.2. DOSIMETRY MODELING

This dose-response assessment begins by expressing doses in common terms to facilitate better comparisons across studies, health effects, species, exposure durations, and exposure routes. Reflecting the importance of metabolism to TCE-induced toxicity, doses are estimated from pharmacokinetic models whenever data are suitable. Otherwise, doses are scaled from animals to humans based on equivalence of $mg/kg^{3/4}$-d (U.S. EPA, 1992). Pharmacokinetic models can account for high- to low-dose nonlinearity and animal-to-human differences in metabolism. The use of pharmacokinetic modeling and dose scaling in developing RfDs is relatively new, representing a move toward harmonizing RfD methods with those already in use for RfCs and cancer assessments.

[53]ATSDR calls these *minimal risk levels* (MRLs), defined as "an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure" (ATDSR, 1997).

4.2.1. Pharmacokinetic Modeling

A pharmacokinetic model for ingested TCE in male mice is described in a state-of-the-science paper by Fisher (2000). The model predicts area-under-the-curve[54] in the liver for TCA and DCA. Compartments[55] include TCE in liver, lung, kidney, fat, gut, rapidly perfused tissues, and slowly perfused tissues. Metabolite submodels include CH, TCA, TCOH, TCOG, and DCA in liver, lung, kidney, and the rest of the body; DCVC and DCVG were not modeled. The model simplifies metabolism by modeling it only in the liver. Model parameters were taken from published literature, measured experimentally, or fitted by running the model with data at 1,200 mg/kg. The model was validated against data at 300, 600, and 2,000 mg/kg, suggesting the ability of the model to make predictions below the dose where it was developed. Parameters were scaled according to body weight. This mouse model was then adapted for inhaled TCE in human males and females. The human model does not include CH and DCA, because CH was not detected in experimental subjects and DCA was detected only intermittently.

Another pharmacokinetic model for ingested and inhaled TCE in male mice, rats, and humans is described in a state-of-the-science paper by Clewell et al. (2000). This model predicts metabolite areas-under-the-curve in plasma. Compartments include TCE in liver, lung, tracheobronchial region, fat, gut, rapidly perfused tissues, and slowly perfused tissues. One-compartment metabolite submodels include TCA, TCOH, TCOG, and DCA. The model also includes descriptions of GST metabolism in the kidney, CH metabolism and clearance in the lung, GST metabolism of TCE to DCVC in the liver, activation of $-lyase and clearance by NAT in the kidney, biliary excretion of TCOG, and enterohepatic recirculation of TCOH. Model parameters were taken from published literature or fitted by running the model with selected data sets. Parameters were scaled allometrically.[56]

Statistical analyses of these pharmacokinetic models are discussed in two additional state-of-the-science papers (Bois, 2000a,b). These analyses use a Bayesian[57] statistical framework and

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[54]*Area-under-the-curve* refers to the tissue concentration of a chemical integrated over time. It is a measure of the long-term average concentration of a chemical in a specific tissue.

[55]Pharmacokinetic models specify *compartments* to represent different tissues or groups of tissues, for example, liver, fat, and the rest of the body.

[56]*Allometric scaling* is a method for calculating equivalent doses across species. It scales volumes according to body weight, blood flow rates and metabolic capacities according to the 3/4 power of body weight, and rate constants according to the inverse 1/4 power of body weight.

[57]*Bayesian* statistical methods regard a model's parameters as random variables with a probability distribution. New data can alter what is thought to be the best distribution for describing each parameter. The distribution based only on prior information and assumptions is called the *prior distribution*. Analysis of new data yields a *posterior distribution* that reconciles the prior information and assumptions with the new data.

Markov-chain Monte-Carlo[58] simulation to refine the models by using more data sets to estimate each model's parameters. The result is a set of calibrated models that better fits a wider range of experimental data. In some cases the calibrated parameters are quite different from the originals, thus, substantial information has been gained by fitting the models to additional experimental data sets. Dose estimates from the calibrated models were used in subsequent state-of-the-science papers (Chen, 2000; Rhomberg, 2000) supporting the cancer assessment, whereas dose estimates from the Clewell et al. (2000) pharmacokinetic model were used in the state-of-the-science paper supporting the RfD and RfC (Barton and Clewell, 2000).

EPA's National Exposure Research Laboratory is developing a Dose Estimating Exposure Model, a model architecture that simulates internal doses for different chemicals and species. As part of this assessment, this model has been configured for TCE, initially following the structure of the Fisher and Clewell models. This new TCE model is being calibrated by comparing its predictions to several experimental data sets. A notable feature of this model is its capacity to simulate simultaneous exposure to TCE, TCA, and DCA. This will be particularly useful in future site-specific applications (for example, at Superfund sites or in assessing the safety of a drinking water supply) where people are exposed to TCE along with TCA and DCA from other solvents or other sources.

4.2.2. Uncertainty Analyses

Another product of the statistical analyses is a quantitative description of uncertainty in the dose estimates (Bois, 2000b). Table 4-1 summarizes distributions of potential dose metrics for modeling liver, lung, and kidney tumors from the calibrated model (Bois, 2000b) on the basis of the Clewell et al. (2000) pharmacokinetic model, the only model to calculate estimates outside the liver. From Table 4-1 it is apparent that lung and kidney dose metrics are quite uncertain, with 95% confidence intervals spanning more than a 5,000-fold range. This may be due to the difficulty in measuring and modeling a transient metabolite such as CH, the possibility that plasma CH may not be a good surrogate for CH accumulated in Clara cells of the lung, or the poorer database for estimating GST metabolites in the kidney. Whatever the reason, this is more uncertainty than is generally present in risk analyses supporting public health decisions. One approach to addressing this uncertainty would be to take lung and kidney dose estimates from the lower end, perhaps the first percentile, of their distributions; this would effectively increase risk

[58]*Markov-chain Monte-Carlo simulation* is an iterative process that can be used within a *Bayesian* statistical framework to (1) sample each model's parameters from their *prior distributions*, (2) fit that model with the sampled parameters to several additional experimental data sets, and (3) compare the model's predictions with the experimental results to obtain *posterior distributions* for the model's parameters that improve the model's fit. These steps are repeated thousands of times until each parameter's *posterior distribution* converges to a more robust distribution that reflects a wider database.

estimates several hundred-fold above the median. Rather than make such a large adjustment, this assessment uses default RfC dosimetry models (U.S. EPA, 1994) when modeling lung tumors by inhalation and the default 3/4-power scaling factor when modeling kidney tumors by ingestion. Liver dose metrics, in contrast, are less uncertain (see Table 4-1); consequently, the cancer assessment uses the calibrated model's median dose estimates for the liver. In this way, uncertainty analysis is used to distinguish between uncertain applications (lung and kidney) and more robust applications (liver), so that pharmacokinetic modeling is used when the results are robust and other methods are considered when there is too much uncertainty.

The statistical analyses also reveal several differences between males and females. Females have a significantly lower alveolar ventilation rate (beyond that explained by allometric scaling), higher TCOH body-to-blood partition coefficient, lower TCA body-to-blood partition coefficient, higher V_{max}/K_m ratio for TCOH glucuronidation, higher conversion of TCOH to TCA, and higher urinary excretion of TCA (Bois, 2000a). These insights are possible because Fisher (2000) collected and modeled data from both males and females. The number of variables with significant differences shows the complexity of overall human variation.

4.2.3. Route Extrapolation

Pharmacokinetic models can be used to investigate questions about extrapolation from one exposure route to another. This can be done by determining oral and inhalation exposures that yield equal internal doses of the active agent.

For this assessment, the pharmacokinetic model developed at EPA's National Exposure Research Laboratory calculated that chronic oral exposure to TCE at 1 mg/kg-d yields similar TCA area-under-the-curve as chronic inhalation exposure to TCE at 75 mg/m^3. This relationship was investigated at several dose levels near 1 mg/kg-d and found to be approximately linear at this dose and below. The relationship is different, however, when matching DCA area-under-the-curve as the internal dose. Chronic oral exposure to TCE at 1 mg/kg-d yields similar DCA area-under-the-curve as chronic inhalation exposure to TCE at 3 mg/m^3 (instead of 75 mg/m^3). This 25-fold uncertainty highlights the importance of further research to identify the appropriate internal dose metric for each toxic effect (see Section 5).

4.3. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

For effects other than cancer, risk assessments have not attempted to describe dose-response curves in the range of environmental exposures. Rather, the focus has been to estimate an exposure level where there is little concern for adverse effects. This RfD is derived through a process of (1) considering all studies and selecting the critical effects that occur at the lowest

dose, (2) selecting a dose (or point of departure[59]) at which the critical effect either is not observed or would occur at a relatively low incidence (for example, 10%), and (3) reducing this dose by uncertainty factors to reflect differences between study conditions and conditions of human environmental exposure.

4.3.1. Critical Effects

Many different toxic effects are associated with oral TCE exposure, as TCE can disrupt fundamental cellular processes through multiple metabolites and mechanisms (see Section 3.4). At higher doses (above approximately 100 mg/kg-d), targets of oral TCE toxicity include the liver, kidney, nervous system, reproductive system, and developing fetus (ATSDR, 1997). Effects have been observed at these sites in acute studies (2 weeks or less), intermediate studies, or chronic studies (1 year or more). At lower doses (below approximately 100 mg/kg-d), there are fewer studies, but effects continue to be observed in several systems, often in subchronic studies. At the lowest doses tested (approximately 1–10 mg/kg-d), effects are observed in the liver, kidney, and developing fetus. These are considered to be the critical effects of oral TCE exposure.

4.3.2. Point of Departure

Table 4-2 summarizes the results of oral studies conducted at the lower doses. To compare studies, doses are expressed in human-equivalent terms. For liver effects, pharmacokinetic modeling (Clewell et al., 2000) was used to estimate plasma TCA as the dose metric (Barton and Clewell, 2000). For kidney effects, statistical analyses (Bois, 2000b) revealed substantial parameter uncertainty in the pharmacokinetic modeling (see Table 4-1), consequently, human-equivalent doses were based on equivalence of $mg/kg^{3/4}$-d (U.S. EPA, 1992). Response levels are presented as either a NOAEL,[60] a LOAEL if the study did not identify a NOAEL, or a modeled LED_{10} if the study results were suitable for modeling (Barton and Clewell, 2000).

Adverse effects have been observed in several studies at a human-equivalent dose range of 1–10 mg/kg-d. For the rat kidney there is a chronic NOAEL at 10 mg/kg-d (Maltoni et al.,

[59]*Point of departure* denotes a dose at the lower end of the observed dose-response curve where extrapolation to lower doses begins. For effects other than cancer, the point of departure is either a NOAEL (no-observed-adverse-effect level), a LOAEL (lowest-observed-adverse-effect level) if no NOAEL can be identified, or a modeled point (for example, an LED_{10} or LED_{01}) if the data are suitable for curve-fitting. For cancer, the point of departure is an LED_{10} (or lower point if one can be reliably estimated) for tumors, or for a key tumor precursor when there is information to describe the mode-of-action.

[60]A *NOAEL* (no-observed-adverse-effect level) is the highest experimental dose without a statistically or biologically significant effect. Note that at this dose there may be effects that are not biologically significant (*i.e.*, not judged *adverse*), or there may be biologically significant effects that are not statistically significant. Statistical significance is affected by study design and sample size, consequently, a NOAEL in one study may show a statistically significant adverse effect in another study with a different design or larger sample.

1986). For the liver there is a subchronic NOAEL at 1 mg/kg-d in mice (Tucker et al., 1982), a subchronic LOAEL at 1 mg/kg-d in mice (Buben and O'Flaherty, 1985), and a subchronic LED_{10} of 0.6 mg/kg-d in rats (Berman et al., 1995). Among these liver values, 1 mg/kg-d is chosen as the point of departure, supported by these three studies as a dose where liver toxicity can begin to be observed in two species after subchronic dosing. For cardiac anomalies in developing rats, there is a LOAEL at 34 mg/kg-d and a NOAEL at 0.05 mg/kg-d (Dawson et al., 1993). The 700-fold difference between this LOAEL and NOAEL reflects the wide dose spacing in this study, which makes use of this endpoint as a point of departure highly uncertain.

4.3.3. Uncertainty Factors

To address differences between study conditions and conditions of lifetime human environmental exposure, the point of departure is reduced by *uncertainty factors*[61] that reflect different areas of uncertainty.

Several studies have identified similar points of departure for adverse liver effects, indicating some degree of confidence in using this effect as a basis for calculating an RfD. There are several sources of uncertainty in using these studies.

(a) *Human variation:* The NOAELs, LOAELs, and LED_{10}s for adverse liver effects (see Section 4.3.2, Table 4-2) were estimated using a pharmacokinetic model (Barton and Clewell, 2000; Clewell et al., 2000). The parameter uncertainty in these modeled dose estimates (estimated between the 50th and 99th percentiles, see Section 4.2.2, Table 4-1) is 15-fold if plasma TCA is used as the dose metric and 20-fold if plasma DCA is used

[61]RfDs apply to lifetime human environmental exposure, including sensitive subgroups. Differences between study conditions and conditions of human environmental exposure may make a dose that appears safe in an experiment not be safe in the environment. *Uncertainty factors* account for differences between study conditions and conditions of human environmental exposure. These include:

(a) *Variation from average humans to sensitive humans:* RfDs apply to the human population, including sensitive subgroups, but studies rarely target sensitive humans. Sensitive humans could be adversely affected at lower doses than a general study population; consequently, general-population NOAELs are reduced to cover sensitive humans.

(b) *Uncertainty in extrapolating from animals to humans:* If an RfD is developed from animal studies, the animal NOAEL is reduced to reflect pharmacokinetic and pharmacodynamic factors that may make humans more sensitive than animals.

(c) *Uncertainty in extrapolating from subchronic NOAELs to chronic NOAELs:* RfDs apply to lifetime exposure, but sometimes the best data come from shorter studies. Lifetime exposure can have effects that do not appear in a shorter study; consequently, a safe dose for lifetime exposure can be less than the safe dose for a shorter period. If an RfD is developed from less-than-lifetime studies, the less-than-lifetime NOAEL is adjusted to estimate a lifetime NOAEL.

(d) *Uncertainty in extrapolating from LOAELs to NOAELs:* RfDs estimate a dose without appreciable risks, but sometimes adverse effects are observed at all study doses. If an RfD is developed from a dose where there are adverse effects, that dose is adjusted to estimate a NOAEL.

(e) *Other factors* to reflect professional assessment of scientific uncertainties not explicitly treated above, including completeness of the overall database, minimal sample size, or poor exposure characterization.

(Bois, 2000b, Rhomberg, 2000). Not known is whether either of these dose metrics is an appropriate indicator of liver toxicity. This pharmacokinetic variation is complemented by pharmacodynamic variation (Renwick, 1998). In the absence of data to quantify human pharmacodynamic variation, a default factor of $10^{1/2}$ [62] has been suggested (Renwick, 1998). Multiplying 15- to 20-fold pharmacokinetic variation by $10^{1/2}$-fold pharmacodynamic variation indicates overall human variation of about 50-fold (rounded to one significant digit).

(b) *Animal-to-human uncertainty:* This aspect of uncertainty can also be viewed in terms of pharmacokinetic and pharmacodynamic components. Animal-to-human pharmacokinetic uncertainty is reflected in the 15- to 20-fold factor just discussed. Animal-to-human pharmacodynamic uncertainty is covered by a default factor of $10^{1/2}$, similar to the practice used for deriving RfCs (U.S. EPA, 1994).

(c) *Subchronic-to-chronic uncertainty:* The NOAEL, LOAEL, and LED_{10} for adverse liver effects (see Section 4.3.2, Table 4-2) were based on subchronic exposures. When subchronic studies are used to derive an RfD for lifetime exposure, an uncertainty factor of up to 10-fold is generally applied. Although duration-response trends are not evident in the animal studies, recent human studies have found statistically significant duration-response trends for decreased testosterone, decreased FSH, decreased SHBG, and increased DHEA (Chia et al., 1997; Goh et al., 1998). Duration-response trends have also been reported for central nervous system toxicity (Rasmussen et al., 1993; Ruitjen et al., 1991). These studies suggest that prolonged exposure to TCE can increase the severity of effects, prompting the use of a partial factor of $10^{1/2}$ until duration-response relationships are better characterized in humans.

(d) *LOAEL-to-NOAEL uncertainty:* The 1 mg/kg-d point of departure is a subchronic LOAEL in one study, a NOAEL in another, and an LED_{10} in a third. When adverse effects are observed at the point of departure, an uncertainty factor of up to 10-fold is generally applied. In this case, a reduced factor of $10^{1/2}$ is appropriate because 1 mg/kg-d appears to be at the boundary where effects can begin to be observed.

(e) *Other factors:* The general population is routinely exposed to many agents that induce CYP2E1, for example, ethanol, acetaminophen, and many ubiquitous environmental contaminants. Moreover, several metabolites of TCE are major environmental contaminants in their own right: for example, TCA and DCA are two principal toxic byproducts of drinking water disinfection. Thus, humans generally have higher background exposures to TCE and its metabolites compared to background exposures in

[62] $10^{1/2}$ denotes the square root of 10. On a logarithmic scale, this represents half of a factor of 10. Numerically, $10^{1/2}$ is approximately equal to 3.1, which is usually rounded to 3 in a final calculation.

test animals; in other words, humans start higher on the dose-response curve than the test animals. To account for this difference between humans and laboratory animals, a modifying factor of $10^{1/2}$ will be applied. This factor appears reasonable in view of the large numbers of people who are at increased risk because of certain medications (for example, acetaminophen and barbiturates) or diseases (for example, diabetes and alcoholism) that affect metabolism and may exacerbate the effects of TCE exposure (Pastino et al., 2000).

This modifying factor to reflect background exposures to TCE and its metabolites is new. It is meant to address the issue of cumulative risks, thus responding to calls by the risk assessment and public health communities that risk assessments would be more realistic and relevant if they address how exposure to other chemicals and stressors can alter a chemical's toxicity. More than for most other chemicals, the general population is exposed to TCE and its metabolites from multiple sources (see Section 3). Consideration of a preexisting background exposure to toxic metabolites is important when these chemicals are assumed to have a sublinear dose-response curve (see Section 1.8, footnote 26). This modifying factor applies only to populations that have background exposure to the metabolites that are involved in TCE's toxicity.

4.3.4. Calculation and Characterization of the Oral Reference Dose

For TCE, an RfD can be based on critical effects in the liver, kidney, and developing fetus. The point of departure is 1 mg/kg-d, a dose where adverse liver effects can begin to be observed in two species after subchronic dosing. A composite uncertainty factor of 5,000 is obtained by multiplying factors of 50 for average-to-sensitive human variation, $10^{1/2}$ for animal-to-human uncertainty, $10^{1/2}$ for using subchronic instead of lifetime studies, $10^{1/2}$ for using a point of departure where adverse effects have been observed, and a modifying factor of $10^{1/2}$ to reflect background exposures to TCE and its metabolites, thus beginning to address the issue of cumulative risks involving TCE. Dividing the 1 mg/kg-d point of departure by a composite uncertainty factor of 5,000 would yield an RfD of 2×10^{-4} mg/kg-d.

When RfDs are calculated using conventional 10-fold uncertainty factors, EPA limits the composite factor to 3,000 when human-equivalent doses are used (U.S. EPA, 1994). With this limitation, the RfD would be 3×10^{-4}. The TCE uncertainty factors are not, for the most part, conventional 10-fold factors: the 50-fold factor for human variation is based on an uncertainty analysis, and the other factors are reduced below 10 based on the available data. Each factor is appropriate at some level, as the RfD is otherwise derived from a dose where adverse effects are observed in subchronic animal studies, but the size of each factor is an open question.

Another perspective on the size of the uncertainty factors is provided by research in progress at ATSDR (El Masri, 2000), in which a data-derived factor covering human variation and animal-to-human uncertainty for TCE was 625 (compared with $50\times10^{1/2}=150$ used in this assessment). ATSDR's analysis showed more uncertainty for TCE than for the three other chlorinated solvents that were modeled. This research suggests that an RfD based on a composite uncertainty factor of 3,000 would leave only a five-fold margin (3,000/625=5) to cover subchronic-to-chronic uncertainty, LOAEL-to-NOAEL uncertainty, and higher cumulative background exposures in humans. This further indicates the high level of uncertainty inherent in any RfD currently derivable for TCE.

Barton and Clewell (2000) suggest an RfD of 0.06–0.12 mg/kg-d based on subchronic liver effects in rats and mice.[63] Their RfD is based on increased liver-weight-to-body-weight ratio, one of the critical effects used here. Their point of departure is equal to the 1 mg/kg-d used here. The difference is their use of an uncertainty factor only for average-to-sensitive human variation. This indicates the potential for discussion of alternative RfDs using fewer uncertainty factors.

The argument for omitting the animal-to-human factor would rest on an assertion that humans are no more sensitive than rats and mice in terms of pharmacodynamic response to TCE. Although some mechanisms of liver toxicity may be quantitatively less in humans (for example, PPAR activation), others might not (for example, cell signaling with DCA). TCE's modes of action are not known, nor is the potential for interaction between modes of action. In addition, critical effects in the kidney and developing fetus occur at doses only slightly above those causing liver toxicity. It has not been established that humans are more sensitive than rats and mice to these other effects.

The argument for omitting the subchronic-to-chronic factor would rest on an assertion that TCE's subchronic effects would not increase over longer durations (the principal studies supporting liver effects involved exposure for only 14 and 30 days). Such short durations usually indicate application of a 10-fold factor. In addition, recent human and animal studies have found statistically significant duration-response trends for several hormone levels, providing further support for using a subchronic-to-chronic factor.

The argument for omitting the LOAEL-to-NOAEL factor would rest on an assertion that the LOAELs for increased liver-weight-to-body-weight ratio represent a minimal effect level. This assertion receives some support from the use of "benchmark dose" modeling, which

[63]Barton and Clewell (2000) suggest an oral RfD in the range of 0.06–0.12 mg/kg-d based on liver effects (increased liver-weight-to-body-weight ratio) in rats (Berman et al., 1995) and mice (Buben and O'Flaherty, 1985), obtaining Weibull-model ED_{10}s of 308–515 mg/kg-d using modeled TCA area-under-the-curve (Clewell et al., 2000) as the dose metric. The corresponding human-equivalent doses were divided by a composite uncertainty factor of 10 (for human variation).

indicates that the LOAELs are comparable to LED_{10}s. Benchmark dose modeling, however, does not mandate rote treatment of LED_{10}s as if they were NOAELs. In this case, the observation of adverse effects at the lowest doses tested after only 14 and 30 days exposure warrants some concern for effects at still lower doses.

This assessment uses partial uncertainty factors of $10^{1/2}$ (approximately threefold) in each of these areas. This is commensurate with the limited nature of the principal studies, in which rats and mice dosed for 14 or 30 days, respectively, showed adverse effects at the lowest tested doses. Further, ATSDR's decision that the database is not strong enough to support a chronic value indicates the presence of much uncertainty in these areas.

Limitations of the database are many. As noted by ATSDR (1997), the chronic-duration studies are problematic. Epidemiologic studies of TCE-contaminated drinking water are limited in their ability to quantify dose-response relationships because of difficulties in estimating past contamination levels and the presence of other solvents with similar metabolic profiles. Most animal studies focus on liver and kidney, organs where TCE induces tumors; studies assessing alteration of organ function or enzyme levels are less common. Finally, neither the occupational studies nor the animal studies provide information about sensitive individuals. In view of these limitations, the use of several uncertainty factors appears reasonable.

4.4. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

4.4.1. Critical Effects

For acute inhalation exposure to high concentrations (above approximately 1,000 ppm, or 5,400 mg/m^3), targets of TCE toxicity include the central nervous system, cardiovascular system, kidney, and developing fetus (ATSDR, 1997). At lower concentrations (approximately 100–1,000 ppm, or 540–5,400 mg/m^3), acute exposure still affects these systems, and there are also intermediate and chronic studies (1 year or more) that show effects on the central nervous system, kidney, liver, and endocrine system (see Section 3.4). At the lowest concentrations, effects are observed in the central nervous system, liver, and endocrine system. These are considered to be the critical effects of inhaled TCE.

4.4.2. Point of Departure

Table 4-3 summarizes the results of inhalation studies of lower concentrations. Concentrations in occupational studies were converted to continuous (24 hr/d, 7 d/wk) concentrations. Concentrations in animal studies were taken from the analysis of Barton and Clewell (2000). If pharmacokinetic-adjusted doses were not available, the RfC methodology (U.S. EPA, 1994) for systemic effects for a water-soluble, perfusion-limited gas was used

instead. Response levels are presented as either a NOAEL, a LOAEL if the study did not identify a NOAEL, or a modeled LEC_{10} if the study results were suitable for modeling.

Adverse effects have been observed in several studies at a concentration range of 5–16 ppm (27–86 mg/m^3). These include LOAELs of 7–16 ppm (38–86 mg/m^3) for signs of central nervous system toxicity in several occupational studies (Rasmussen et al., 1993; Ruitjen et al., 1991; Vandervort et al., 1973; Okawa and Bodner, 1973). Also, an LEC_{10} of 5 ppm (27 mg/m^3) was identified for heart rate and electroencephalographic changes in rats (Arito et al., 1994). For endocrine effects, a LOAEL of 11 ppm (59 mg/m^3) was identified for decreased mean levels of testosterone, decreased FSH, decreased SHBG, and increased DHEA in an occupational study (Chia et al., 1997; Goh et al., 1998). A pharmacokinetic-model-derived LEC_{10} of 5 ppm (27 mg/m^3) was identified for increased liver-weight-to-body-weight ratio in mice (Kjellstrand et al., 1983).

Together, these studies indicate 5–16 ppm (27–86 mg/m^3) as the lower end of the range of concentrations at which adverse effects have been observed. From this range, 7 ppm (38 mg/m^3) is chosen as the point of departure. This concentration was identified as a subchronic LOAEL for central nervous system effects in two occupational studies and is supported by central nervous system effects in rats at 5 ppm (27 mg/m^3). This latter dose is also associated with a 10% increased incidence of liver effects in mice.

4.4.3. Uncertainty Factors

To address differences between study conditions and conditions of human environmental exposure, the point of departure is reduced by several uncertainty factors.

(a) *Human variation:* The occupational studies observed healthy adult workers, who do not reflect the potential for effects in children, the elderly, or those with disease or other conditions that can increase susceptibility. This supports use of the standard 10-fold factor for human variation. If human variation for this effect were as large as that for liver effects (see Section 4.3.3), then this factor would be an underestimate.

(b) *Animal-to-human uncertainty:* Because the point of departure is supported by human studies, this factor is not needed.

(c) *Subchronic-to-chronic uncertainty:* The point of departure is based on subchronic studies. Duration-response trends have been reported for central nervous system toxicity (Rasmussen et al., 1993; Ruitjen et al., 1991). These observations suggest that continuing exposure to TCE can increase the severity of effects, supporting a 10-fold factor to address the potential for more severe toxicity from lifetime exposure to TCE. Statistically significant duration-response trends have also been observed for decreased mean levels of testosterone, decreased follicle-stimulating hormone, decreased SHBG,

and increased DHEA in humans (Chia et al., 1997; Goh et al., 1998), plus decreased testosterone in rats (Kumar et al., 2000).

(d) *LOAEL-to-NOAEL uncertainty:* The central nervous system effects and the endocrine effects observed in occupational studies are LOAELs, supporting a 10-fold factor to approach the range where a NOAEL could be expected.

(e) *Other factors:* These are not being used.

4.4.4. Calculation and Characterization of the Inhalation Reference Concentration

For TCE, an RfC can be based on critical effects in the central nervous system, liver, and endocrine system. The point of departure is 7 ppm (38 mg/m^3). This concentration was identified as a subchronic LOAEL for central nervous system effects in two occupational studies and is further supported by central nervous system effects in rats and liver effects in mice at 5 ppm (27 mg/m^3). A composite uncertainty factor of 1,000 is obtained by multiplying factors of 10 for average-to-sensitive human variation, 10 for starting from subchronic instead of lifetime studies, and 10 for starting from effect levels instead of NOAELs. Dividing the 38 mg/m^3 point of departure by a composite uncertainty factor of 1,000 yields an RfC of 4×10^{-2} mg/m^3.

Barton and Clewell (2000) suggest an RfC of 0.4–1 ppm (2–5 mg/m^3) based on subchronic neurological effects in rats.[64] Their RfC is based on one of the studies supporting the point of departure. The difference is their use of an uncertainty factor for only average-to-sensitive human variation. This indicates the potential for discussion of alternative RfCs that do not incorporate uncertainty factors for using a subchronic instead of a lifetime study or for using an effect level instead of a NOAEL.

4.4.5. Comparison of Reference Dose and Reference Concentration

The similarity of effects by oral or inhalation exposure to TCE suggests using route extrapolation (see Section 4.2.3):

(a) to compare effect levels for oral and inhalation exposure, and

(b) to compare the RfD and RfC.

The results will depend on the equivalence between oral dose and inhaled concentration that yields similar internal levels of the active agent causing a particular toxic effect. If TCA area-under-the-curve is the appropriate dose metric, then ingesting TCE at 1 mg/kg-d yields similar internal TCA as inhaling TCE at 75 mg/m^3. On the other hand, if DCA area-under-the-

[64]Barton and Clewell (2000) suggest an inhalation RfC in the range of 0.4–1 ppm (2–5 mg/m^3) based on neurologic effects (changes in sleep and wakefulness) in rats (Arito et al., 1994), obtaining an ED_{10} of 0.57 ppm using modeled TCOH in blood (Clewell et al., 2000) as the dose metric. The corresponding human-equivalent concentration was divided by a composite uncertainty factor of 10 (for human variation).

curve is the appropriate dose metric, then ingesting TCE at 1 mg/kg-d yields similar internal DCA as inhaling TCE at 3 mg/m^3 (see Section 4.2.3).

The oral point of departure is an equivalent human dose of 1 mg/kg-d (see Section 4.3.2). This corresponds to an inhaled concentration of 75 mg/m^3 if TCA is the appropriate dose metric for route extrapolation, and 3 mg/m^3 if DCA is the appropriate dose metric. The 25-fold range between these two concentrations contains the inhalation point of departure of 38 mg/m^3 (see Section 4.4.2). This suggests a convergence of effect levels for these two exposure routes.

Similarly, the oral RfD is 3×10^{-4} mg/kg-d (see Section 4.4.4). This corresponds to an inhaled concentration of 2×10^{-2} mg/m^3 if TCA is the appropriate dose metric and 9×10^{-4} mg/m^3 if DCA is the appropriate dose metric.[65] The inhalation RfC of 4×10^{-2} mg/m^3 is close to the concentration that results when TCA is used as the dose metric.

Another useful route extrapolation compares TCE exposures in the occupational and drinking water studies. In the Singapore cohort (Chia et al., 1997; Goh et al., 1998), hormone disruption was observed in male workers exposed to an average of 30 ppm (162 mg/m^3) for 8 hr/d. This inhalation exposure yields similar internal TCA as ingesting TCE at 0.71 mg/kg-d, and this dose corresponds to a drinking water concentration of 25 mg/L for a 70-kg adult drinking 2 L/d.[66] The corresponding ingested dose (0.71 mg/kg-d) is close to the oral point of departure (1 mg/kg-d), reiterating the convergence between the oral and inhalation databases and giving them greater confidence than they would have if analyzed separately. At the same time, the corresponding drinking water concentration (25 mg/L) greatly exceeds the Safe Drinking Water Act maximum contaminant level of 5 ：g/L for TCE, suggesting that there is much value in attempting to use drinking water studies to estimate risk and avoid some of the problems of low-dose extrapolation (see Section 4.5.1).

4.5. SLOPE FACTOR AND INHALATION UNIT RISK FOR CANCER

Cancer risk assessments have described dose-response curves in the range of environmental exposures, conveying an appreciation of how risk decreases as dose decreases. Past risk assessments have used a dose-response curve that is linear at low doses, implying that

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[65]The calculations are:

$(2\times10^{-4}$ mg/kg-d) × (75 mg/m^3)/(mg/kg-d) = 1.5×10^{-2} ：g/m^3

$(2\times10^{-4}$ mg/kg-d) × (3 mg/m^3)/(mg/kg-d) = 6×10^{-4} ：g/m^3

[66]The calculations are:

(162 mg/m^3) × (8 hr/24 hr) × (1 mg/kg-d)/(75 mg/m^3) = 0.71 mg/kg-d

(0.71 mg/kg-d) × (70 kg) / (2 L/d) = 25 mg/L

risk decreases proportionately with dose.[67] The slope of the dose-response curve at low doses is called a *slope factor*.

EPA's proposed cancer guideline revisions (U.S. EPA, 1996) describe a two-step process of modeling the observed data followed by extrapolation to lower doses. Extrapolation to lower doses follows either a linear or a nonlinear approach, depending on what is known about mode-of-action. Extrapolation begins from a *point of departure* near the lowest doses. When linear extrapolation is used, the slope factor is determined by the line from the point of departure toward zero. When the mode-of-action is understood well enough to support nonlinear extrapolation, a dose-response curve is not estimated below the point of departure. Instead, a discussion provides information about the distance from the point of departure to a dose where there would be little concern for cancer.

The use of pharmacokinetic and dosimetry models carries an implicit assumption of tumor site concordance across species; therefore, it is important to assess and consider each tumor site that may be relevant to human environmental exposure. In this regard, it is important to note that lack of suitable dose-response data has precluded development of slope factors for some sites where there might be a cancer risk, notably for cervical cancer and prostate cancer.

4.5.1. Risk Estimates From Cancer Epidemiology Studies

Quantitative risk methods for human studies must be tailored to the type of data available in each particular case. Of utmost importance is the availability of quantitative exposure data, which can be the principal determinant of the quality of the risk estimates.

4.5.1.1. *Finnish Cohort*

One set of risk estimates was derived from a cohort of 2,050 male and 1,924 female Finnish workers exposed to TCE and other solvents (Anttila et al., 1995). This cohort had statistically significant increases in non-Hodgkin's lymphoma and, among workers exposed for more than 20 years, liver cancer. Although kidney cancer was not statistically significantly increased in this cohort, the observed incidence was used to bound the potential risk identified qualitatively from the epidemiologic studies as a whole (Wartenberg et al., 2000). For this

[67]Low-dose-linear models are appropriate for extrapolation to lower doses when a carcinogen acts in concert with other exposures and processes that cause a background incidence of cancer (Crump et al., 1976; Lutz, 1990). Further, even when the mode-of-action indicates a nonlinear dose-response curve in homogeneous animal populations, the presence of genetic and lifestyle factors in a heterogeneous human population tends to make the dose-response curve more linear (Lutz, 1990). This is because genetic and lifestyle factors contribute to a wider spread of human sensitivity, which extends and straightens the dose-response curve over a wider range. Although these considerations provide a reasonable argument for a model that is linear at low doses, the relation of the low-dose slope to one from the experimental range is uncertain; this uncertainty increases with the distance from the experimental range.

assessment, Anttila made available to EPA urinary TCA measurements and the cancer status for each worker.[68] A separate study of 51 male workers (Ikeda, 1972) was used to derive a relationship between urinary TCA and air concentrations of TCE:

Urinary TCA (mg/L) = 2.956 × Ambient TCE (ppm, 8 hr/d, 6 d/wk)

Occupational exposure duration, which was not available, was assumed to be 15 years, and each worker's mean urinary TCA measurement was taken as representative for this duration. The resulting inhalation unit risk estimates, adjusted for lifetime exposure to an average of 1 ug/m^3 of TCE in air, are:

Liver cancer:	1×10^{-7}; upper bound, 9×10^{-7}
Kidney cancer:	2×10^{-5}; upper bound, 3×10^{-5}
Non-Hodgkin's lymphoma:	5×10^{-5}; upper bound, 9×10^{-5}
Overall:	7×10^{-5}; upper bound, $>9\times10^{-5}$

These estimates are based on a small number of cancer cases. As with other occupational studies, they are based on healthy adult workers and may not be representative of children or other groups. Moreover, the exposure duration is uncertain. In addition, the workers were exposed to other solvents (albeit solvents with similar metabolic pathways, potential modes of action, and toxic effects), so that attributing all risk to TCE can overestimate the risk from TCE (though not, perhaps, for the combined solvent exposure). On the other hand, the exposure metric is based on biological measurements of a major metabolite of TCE.

4.5.1.2. *Hill Air Force Base Cohort*

Another analysis considered the cohort of Hill Air Force Base workers exposed to TCE and other chemicals (Blair et al., 1998). This cohort showed nonsignificant increases in mortality from liver cancer, kidney cancer, and non-Hodgkin's lymphoma, cancers that are elevated in the epidemiologic studies overall (Wartenberg et al., 2000). Blair made available to EPA a qualitative exposure index and the cancer mortality status for each worker. The exposure index did not quantify exposure, instead, it classified each job as either high or low peak exposure. Difficulties in converting this exposure index into a credible quantitative estimate include differences between peak exposure and cumulative exposure and the diversity of jobs and exposure levels in the cohort; indeed, Blair (1998) cautioned that the exposure index was not

[68]The data for each cancer case are as follows:

	Liver cancer				Kidney cancer						Non-Hodgkin's lymphoma							
Average urinary TCA (: mol/L)	15	257	17	112	92	23	77	363	2,217	3	3	48	207	312	422	13	44	21
Age at first TCA measurement	64	50	29	15	52	48	41	40	32	54	54	36	63	34	33	33	18	21
Age at last TCA measurement	65	52	30	25	54	49	42	41	33	55	55	37	64	35	34	34	19	27
Age at end of followup	78	79	56	41	68	62	62	63	49	80	74	61	81	58	62	57	39	43

convertible into ppm. Without a quantitative estimate of exposure, it is not possible to derive risk estimates.

4.5.1.3. *New Jersey Drinking Water Study*

Risk estimates were derived from a study of drinking water contamination in a 75-town area of New Jersey (Cohn et al., 1994). Female residents had increased incidences of leukemia (RR=1.4, 95% CI=1.1–1.9, N=56) and non-Hodgkin's lymphoma (RR=1.4, 95% CI=1.1–1.7, N=87). For homes exceeding the Safe Drinking Water Act maximum contaminant level of 5 µg/L, the average concentration was 23 ∶g/L and the highest concentration was 67 µg/L. Using this average from the most-exposed homes, a drinking water unit risk estimate for non-Hodgkin's lymphoma from exposure to 1 ∶g/L of TCE in drinking water is:

$$(RR - 1) \times \text{Background risk} / \text{Average concentration} =$$

$$(1.4 - 1) \times (6\times10^{-4}) / (23\ \text{∶g/L}) = 1\times10^{-5} \text{ per ∶g/L}$$

where 6×10^{-4} is the background risk of non-Hodgkin's lymphoma in the United States. This unit risk can be converted to a slope factor by multiplying by 35,000 (based on a 70-kg adult drinking 2 L/d). The resulting slope factor is 3.5×10^{-1} per mg/kg-d average lifetime exposure to TCE. The residents were exposed to other drinking water contaminants, so that attributing all risk to TCE can overestimate the risk from TCE. On the other hand, this risk estimate is derived from human environmental exposure, avoiding the uncertainties of animal-to-human extrapolation and high- to low-dose extrapolation. Moreover, using the average concentration from the most-exposed homes would tend to overestimate exposure and, hence, underestimate unit risks.

4.5.1.4. *German Cohort*

The California Environmental Protection Agency (Cal/U.S. EPA, 1999) derived a slope factor from the increased incidence of kidney cancer in German cardboard workers exposed to TCE (Henschler et al., 1995). In the absence of exposure data, the German threshold limit value of 50 ppm (270 mg/m^3) was used as a surrogate for the average workplace concentration. This was converted to a lifetime average daily dose, assuming 50% absorption of inhaled TCE:

$$50 \text{ ppm} \times (8 \text{ hr}/24 \text{ hr}) \times (5 \text{ d}/7 \text{ d}) \times (15.2 \text{ yr}/70 \text{ yr}) \times 0.50 \times 5.37 \text{ (mg/m}^3\text{)/ppm}$$

$$\times\ 20 \text{ m}^3\text{/d} / 70 \text{ kg} = 1.98 \text{ mg/kg-d}$$

From the reported kidney cancer incidence (7/169, SIR=13.53 based on East German background rates), a slope factor was obtained by the following calculation, based on an additive risk model that is linear in dose:

$$(7/169) \times (1–1/13.53) / 1.98 \text{ mg/kg-d} = 1.9 \times10^{-2} \text{ per mg/kg-d}$$

By this calculation, Cal/EPA converted the air concentration into mg/kg-d by figuring gross amount inhaled by a 70-kg adult breathing 20 m^3/d. Therefore, to convert the slope factor to an inhalation unit risk, 20 m^3/d and 70 kg would be removed from the preceding calculations. The resulting inhalation unit risk is 5×10^{-6} per ⋮ g/m^3.

To the extent that the workers were exposed to mixed solvents and other risk factors, this slope factor derived from the Henschler study would tend to overestimate risks from TCE, because it attributes all kidney cancers to TCE and not to the other risk factors. On the other hand, to the extent that the threshold limit value of 50 ppm overestimates average workplace concentration, this slope factor would tend to underestimate risks. Further uncertainty arises from using an unadjusted incidence (7/169) instead of one based on life-table methods, from comparing this incidence with East German background rates (the plant was in West Germany, but West German rates were unavailable), and from treating exposure concentration and exposure duration as constant across all workers.

Variation in human susceptibility was explored by further analyses based on observed differences in kidney cancer risk attributed to GST polymorphisms (Brüning et al., 1997). The odds ratio for the combined genotype GSTM/GSTT (found in 40% of Caucasians) was 3.75. This corresponds to a higher slope factor (3.5×10^{-2} per mg/kg-d) for those with the combined genotype and a lower slope factor (9×10^{-3} per mg/kg-d) for the rest of the population (Cal/U.S. EPA, 1999).

4.5.2. Risk Estimates From Liver Tumors in Mice

TCE causes hepatocellular carcinomas and adenomas in male and female mice exposed by ingestion or inhalation (see Table 3-1). TCE is also associated with liver cancer in humans (Wartenberg et al., 2000). Several approaches are explored for estimating these risks.

4.5.2.1. *Mechanism-Based Modeling*

Chen (2000) developed a mechanism-based dose-response model for mouse liver tumors, based on mechanistic hypotheses discussed by Bull (2000). The model is a stochastic form of the two-stage model, in which normal cells can become initiated (the first stage); initiated cells either die, proliferate into a clone of initiated cells, or are converted to malignancy (the second stage); and malignant cells either die or progress to a detectable tumor. Dose metrics were area-under-the-curve of TCA or DCA in the liver, taken from Bois's calibration of Fisher's pharmacokinetic model (Bois, 2000a). Chen's model assumes that TCE induces liver tumors through TCA and DCA acting by clonal expansion of preexisting initiated cells. This assumption of action on only

preexisting initiated cells implies that TCE-induced liver tumor incidences are proportional to background liver tumor incidences.

In a sequence of applications of the mechanism-based model, (1) a dose-response curve for DCA-induced liver tumors was developed from bioassays of DCA in mice, (2) this curve was found to be consistent with bioassays of TCA in mice, considering the rate at which some TCA is metabolized to DCA, and (3) these curves were found to be consistent with the bioassays of TCE in mice, considering the rate at which some TCE is metabolized to TCA and DCA (Chen, 2000). This suggests that TCE produces enough DCA to theoretically explain the mouse liver tumors induced in the TCE bioassays, though it cannot rule out a role for TCA or other metabolites. Thus, the relative importance of DCA or TCA in TCE-induced mouse liver tumors is not resolved by mechanism-based modeling.

The mechanism-based model also explored extrapolation to lower doses. Following the model below the observed range yields an (upper bound) unit risk of 2.4×10^{-8} for oral exposure to 1 ug/L of TCE in drinking water (Chen, 2000).

4.5.2.2. *Empirical Modeling*

Rhomberg (2000) fitted empirical dose-response models to the liver tumor data. Dose metrics were area-under-the-curve of TCA or DCA in the liver or plasma, taken from Bois's calibrations of Fisher's and Clewell's pharmacokinetic models (Bois, 2000a,b). Table 4-4 presents the modeled LED_{10}s that provide a point of departure for low-dose extrapolation. These LED_{10}s reveal substantial model uncertainty between Fisher's and Clewell's models. They also reveal that Bois's calibrations bring some convergence: harmonic means[69] of LED_{10}s from the four experiments span 0.16–25.82 mg/kg-d when dose estimates come from Fisher's and Clewell's original models, but narrow to 0.5–3.1 mg/kg-d when dose estimates come from Bois's calibrations of those models. (Bois did not calibrate Fisher's model with DCA as the dose metric; in this case, only Fisher's original model is considered.) This latter range (0.5–3.1 mg/kg-d) is used as the point of departure for low-dose extrapolation. Although both endpoints of this range use TCA as the dose metric, this does not imply a preference for TCA over DCA. The DCA range (1.7–2.5 mg/kg-d) simply falls within the TCA range, moreover, Section 4.5.1 discusses why neither is established as the appropriate dose metric.

It is tempting to note that the point-of-departure range for liver tumors (0.5–3.1 mg/kg-d) includes the point of departure for liver toxicity (a subchronic LOAEL of 1 mg/kg-d for increased liver-weight-to-body-weight ratio; see Section 4.3.2). This concordance, however, is likely to be

[69]The *harmonic mean* is computed as $N/(\sum 1/X_i)$. For this application, this is equivalent to averaging slope factors (which are inversely proportional to LED_{10}s), then taking the reciprocal to convert the average slope factor back to a dose.

only coincidental, as liver weight change is a nonspecific effect observed in both rats and mice, but rats have not developed liver tumors from TCE exposure. The liver weight changes, therefore, are not considered a key event in liver tumor development.

4.5.2.3. *Extending the Observed Range Using Subchronic-dosing Studies*

A key determinant of risk is the shape of the dose-response curve below the point of departure. One source of information about lower doses is the comprehensive study by Maltoni et al. (1986), who had the foresight to investigate different exposure durations in parallel experiments. Groups of Swiss mice inhaled TCE at 100–600 ppm (540–3,240 mg/m^3), 7 hours daily, 5 days weekly, for either 8 weeks or 78 weeks; then the experiments continued past 2 years to ensure that the latent period for tumor development had passed.

Table 4-5 and Figure 4-1 show the incidence of hepatomas in male mice (females did not develop hepatomas). If response is considered as a function of cumulative exposure (concentration times duration), the 8-week exposure groups can be regarded as extending the observed range to lower cumulative exposures. Overall, response decreases as cumulative exposure decreases, but the curve does not appear to be sublinear anywhere in this extended range. This suggests that exposures must be far below the observable range before the risk drops appreciably.

This finding may be pertinent for risk assessments of less-than-lifetime exposure durations. If the lifetime average dose is low because the exposure duration is relatively short, it may be important to consider that risk may not fall proportionately as exposure duration decreases. That is, cumulative exposure (concentration times duration, or area-under-the-curve) may not be a good dose metric to use to predict liver tumor risk.

4.5.2.4. *Extending the Observed Range Using DCA and TCA Studies*

Another source of information at lower doses are the studies of DCA- or TCA-induced liver cancer. Bioassays of these TCE metabolites cover a 100-fold dose range, wider than the dose range of the TCE bioassays. Chen (2000) analyzed these studies jointly, using area-under-the-curve of DCA in the liver as a common dose metric.

Table 4-6 and Figure 4-2 bring together the dose-response information from the DCA, TCA, and TCE bioassays. The DCA and TCA studies extend the dose range as much as 100-fold below the TCE studies. Overall, the responses appear to decrease as dose decreases, but the dose-response curve does not appear to be sublinear anywhere in this extended range, again suggesting that exposures must be far below an LED_{10} before the risk drops appreciably.

Looking at the multiple-dose TCA and DCA bioassays individually, the dose-response curve for TCA appears linear, whereas the curve for DCA appears sublinear. If we consider

tumor multiplicity along with tumor incidence, however, the DCA dose-response curve, too, becomes linear (DeAngelo et al., 1999). Tumor multiplicity may more closely reflect rates of underlying carcinogenic processes.

4.5.2.5. *Extrapolation to Lower Doses*

Under EPA's proposed cancer guideline revisions (U.S. EPA, 1996), choice of linear or nonlinear extrapolation to lower doses is based on what is known about the mode-of-action. The evidence indicates that TCE-induced liver tumors arise through multiple metabolites and multiple modes of action (see Section 3.5.1). The preponderance of evidence suggests that the CYP450 metabolites are sufficient to explain the liver tumors (Bull, 2000; Chen, 2000). CH and TCA cause liver tumors in mice, and DCA causes liver tumors in mice and rats. A plausible mode-of-action is that TCE induces liver tumors through TCA and DCA modifying cell signaling systems that control rates of cell division and cell death (Bull, 2000). Tumors from the TCA and DCA bioassays arise from different mechanisms and can be distinguished: TCA-induced tumors are associated with peroxisome proliferation, while DCA-induced tumors are associated with selectively inhibiting growth of normal hepatocytes and suppressing apoptosis (Bull, 2000). DCA may also have different modes of action at different doses, possibly involving differential accumulation of glycogen in normal and initiated liver cells (Bull, 2000). Moreover, DCA is also weakly mutagenic (Moore and Harrington-Brock, 2000), although this property is not needed to explain the bioassay results. Characterizing the tumors from the TCE bioassays is a critical research need that presently limits understanding of the respective roles of TCA and DCA in TCE-induced tumors (see Section 5). At present, however, the extensive mode-of-action information still lacks identification of the sequence of key events and a quantitative description of the doses at which those key events begin to occur. In such cases, EPA's proposed cancer guideline revisions (U.S. EPA, 1996) support consideration of both linear and nonlinear extrapolation to lower doses.

Linear extrapolation follows a straight line toward the origin, the slope of this line (or *slope factor*) is $0.10/LED_{10}$. The LED_{10} range of 0.5–3.1 mg/kg-d yields slope factors of 3×10^{-2} to 2×10^{-1} per mg/kg-d. Because the pharmacokinetic models allow for a nonlinear relationship between (external) exposure and (internal) dose, this "straight-line" extrapolation can reflect nonlinearity in the exposure-dose-response data.

Nonlinear extrapolation under the proposed cancer guideline revisions (U.S. EPA, 1996) does not attempt to describe dose-response curves in the range of environmental exposures, instead, it examines whether an exposure is small enough to pose little risk of cancer. The goal is that exposures be small enough that "the key events in tumor development would not occur among sensitive individuals in a heterogeneous human population, thus representing an actual

'no effect level'" (U.S. EPA, 1999). A nonlinear analysis considers several factors[70] where the available data may fall short of this ideal.

(a) *Nature of the response:* The point of departure is ideally based on key precursor events. If tumors are used instead, then the dose is adjusted to bring it into the range where the key precursor events would not occur (U.S. EPA, 1999). For TCE-induced liver cancer, further research is needed to identify the key events and the doses at which the key events begin to occur (see Section 5). These doses will be below the LED_{10}s where tumors are clearly observable, how far below cannot now be determined.

(b) *Shape of the observed dose-response curve:* The shape of the dose-response curve can indicate how fast risk decreases below the point of departure. For TCE-induced liver cancer, dose-response curves are ambiguous, consistent with either low-dose linearity or nonlinearity (Rhomberg, 2000). Extending the dose-response curve using information from subchronic-dosing studies or from DCA and TCA bioassays suggests that the dose-response curve may remain linear quite far below, perhaps two orders of magnitude below, the LED_{10}s. At still lower doses, the mode-of-action may ultimately be nonlinear; nonetheless, current data do not identify how far below the LED_{10}s sublinearity begins to prevail. Further research is needed to identify the key precursor events and describe their dose-response curves (see Section 5).

(c) *Human sensitivity compared with experimental animals:* This factor has been viewed as having pharmacokinetic and pharmacodynamic components (U.S. EPA, 1994). For TCE, the pharmacokinetic component is addressed by the pharmacokinetic models, which predict higher internal doses of TCA and DCA in humans than in mice. Little information is typically available on the pharmacodynamic component, and the proposed cancer guideline revisions (U.S. EPA, 1996) recommend that humans be considered

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[70]A nonlinear analysis considers the following factors (U.S. EPA, 1999):

(a) *Nature of the response:* The point of departure is ideally based on key precursor events, not tumors, just as RfDs and RfCs are based on critical (precursor) effects occurring at lower doses than those associated with frank toxicity. Precursor events are likely to occur at doses below those where tumors are observable. If a point of departure is based on tumors, then the point of departure is adjusted to reflect the dose at which key precursor events would begin to occur.

(b) *Shape of the observed dose-response curve:* If the dose-response curve is steep at the point of departure, then the occurrence of key events declines rapidly in this range. On the other hand, if the dose-response curve is relatively shallow, then the point where the key event no longer occurs may lie far below this range.

(c) *Human sensitivity compared with experimental animals:* If the point of departure is developed from animal studies, this dose is adjusted to reflect pharmacokinetic and pharmacodynamic factors that may make humans and animals differ in sensitivity.

(d) *Nature and extent of human variability in sensitivity:* Pertinent information would come from human studies, because animal studies, particularly those using homogeneous animal strains, do not provide information about human variation.

(e) *Human exposure:* Cancer response can depend on the expected pattern of human exposure, including the magnitude, frequency, and duration of exposure.

potentially more sensitive by a default factor of $10^{1/2}$. For TCE, however, the prevailing view is that humans would be quantitatively less sensitive than mice to the PPAR activity and other processes leading to the mouse liver tumors (Bull, 2000). In this case, an inverse factor of $10^{-1/2}$ could be used to reflect the potential lesser sensitivity of humans (U.S. EPA, 1996).

(d) *Nature and extent of human variability in sensitivity:* For TCE, this factor can be based on the statistical uncertainty analyses (Bois, 2000b). A factor of 15–20 reflects the pharmacokinetic uncertainty in the liver between the 50th and 99th percentiles (see Table 4-1). Coupled with a factor of $10^{1/2}$ to cover pharmacodynamic differences between humans, a factor of 50–60 would appear appropriate to reflect human variation in sensitivity.

This discussion of data-derived factors indicates several large uncertainties: 50–60-fold to reflect human variation, approximately 100-fold to reach a range where the dose-response curve does not appear linear in some bioassay, an as-yet-undeterminable factor to reflect the difference in dose between tumors and key precursor events, and a factor of $10^{-1/2}$ to reflect the potential for humans to be less sensitive than mice. Overall, then, the composite factor would appear comparable to the 3000–10,000 that is the maximum for developing a credible RfC or RfD (U.S. EPA, 1994; Barnes and Dourson, 1988).

4.5.2.6. *Comparison of Empirical and Mechanism-Based Modeling Results*

Mechanism-based modeling yields a unit risk of 2.4×10^{-8} for oral exposure to 1 μg/L in water (Chen, 2000). This unit risk can be converted to a slope factor by multiplying by 35,000 (based on a 70-kg adult drinking 2 L/d). The resulting slope factor is 8.4×10^{-4} per mg/kg-d. This result lies below the empirically based slope factor range of 3×10^{-2} to 2×10^{-1}.

The mechanism-based slope factor includes a 100-fold reduction attributable to an assumption that chemically induced tumor rates scale across species in proportion to background tumor rates, the rate in mice being 100 times that of humans. This difference is not based on any data for TCE, rather, it is a corollary of the model's assumptions that TCE acts by clonal expansion of preexisting initiated cells and that there is strict tumor site concordance across species. If these assumptions, which generally are not used in cross-species extrapolations for other chemicals, were not used here, then the mechanism-based slope factor would be 100-fold higher, or 8×10^{-2} per mg/kg-d. This latter result lies within the slope factor range based on empirical modeling.

4.5.3. Risk Estimates From Lung Tumors in Mice

4.5.3.1. *Modeling*

TCE causes lung adenomas and adenocarcinomas in mice exposed by inhalation (see Table 3-5). Pharmacokinetic models (Clewell et al., 2000; Bois, 2000b) were used to estimate area-under-the-curve and peak concentration of CH in plasma, plasma CH being considered as a surrogate for CH accumulation in Clara cells of the lung. Bois's uncertainty analysis (Bois, 2000b), however, revealed the presence of substantial parameter uncertainty in the lung dose estimates (see Table 4-1, Section 4.2.2). Consequently, dosimetry models for deriving inhalation RfCs were used instead (U.S. EPA, 1994). Rhomberg (Rhomberg, 2000) fitted empirical dose-response models to the lung data (see Table 4-7). The resulting LEC_{10}s are quite similar, ranging from 1.3×10^{5} to 3.6×10^{5} $\mu g/m^3$.

4.5.3.2. *Extrapolation to Lower Doses*

The proposed mode-of-action for TCE-induced lung tumors is that rapid CYP2E1 metabolism of TCE in the Clara cells of the mouse lung leads to an accumulation of CH and causes cell damage and compensatory cell replication that leads to tumor formation (Green, 2000). Because humans have fewer Clara cells and little CYP2E1 activity in the lung as a whole, humans may be less sensitive to this particular mode-of-action. (This argument, however, pertains more to the relative sensitivity of mice and humans than to nonlinearity in either mice or humans.) Although this hypothesis is consistent with the higher level of CYP2E1 in mouse lung, it has not yet been determined whether accumulation of CH is a key event causing tumors or a coincidental event unrelated to tumors. Moreover, several questions about this hypothesis remain unresolved (see Section 3.5.3). In addition, CH is clearly clastogenic and mutagenic at high doses (Moore and Harrington-Brock, 2000), raising the possibility of multiple modes of action. For these reasons, this assessment will pursue both linear and nonlinear extrapolation to lower doses.

Linear extrapolation takes the form of a straight line to the origin; the slope of this line (or *inhalation unit risk*) is $0.10/LEC_{10}$. The LEC_{10} range of 1.3×10^{5} to 3.6×10^{5} $\mu g/m^3$ yields unit risks of 3×10^{-7} to 8×10^{-7} per $\mu g/m^3$.

Nonlinear extrapolation under the proposed cancer guidelines (U.S. EPA, 1996) considers several factors in identifying a dose where there would be little concern for cancer.

(a) *Nature of the response:* The proposed key event, CH accumulation in Clara cells, has been studied only at high doses (450 ppm and above, equivalent to 2,430 $\mu g/m^3$) (Green, 2000) relative to those where lung tumors have been observed (150–300 ppm, or 810–1,620 mg/m^3). This makes determination of cause-and-effect premature, and it does not identify doses at which CH accumulation would not occur. Further research is needed

to establish the quantitative relationship between CH accumulation and lung tumors so that a point of departure can be based on the key precursor event.

(b) *Shape of the observed dose-response curve:* Dose-response curves for TCE-induced lung cancer are ambiguous, consistent with either linearity or nonlinearity at low doses (Rhomberg, 2000). Current data do not identify how far below the LEC_{10}s sublinearity begins to prevail. Further research is needed to link CH accumulation to lung tumors and describe the dose-response curve for CH accumulation.

(c) *Human sensitivity compared with experimental animals:* The pharmacokinetic component is addressed by inhalation dosimetry models (U.S. EPA, 1994). For the pharmacodynamic component, if rapid CYP2E1 metabolism leading to CH accumulation in Clara cells is the key event, humans may be quantitatively less sensitive than mice to this effect (Green, 2000). In this case, an inverse factor of $10^{-1/2}$ could be used to reflect the potential lesser sensitivity of humans (U.S. EPA, 1996). Nonetheless, humans may not necessarily be less sensitive if other CYP450 enzymes play a role or if other lung cell types are involved.

(d) *Nature and extent of human variability in sensitivity:* For TCE in the lung, this factor is problematic in light of the substantial variation revealed by the uncertainty analysis of the pharmacokinetic modeling (Bois, 2000b). A factor of more than 300 would be needed to reflect just the pharmacokinetic uncertainty in the lung between the 50th and 99th percentiles (see Table 4-1). A further factor would be needed to reflect pharmacodynamic differences. Clearly, more research is needed in the area of human variation.

In light of these research needs (see Section 5), it is difficult to proceed with nonlinear extrapolation to establish a dose at which lung tumors would not be of concern.

4.5.4. Risk Estimates From Kidney Tumors in Rats

4.5.4.1. *Modeling*

TCE causes kidney adenocarcinomas and adenomas in male and female rats by oral gavage and in male rats by inhalation (see Table 3-2). TCE is also associated with kidney cancer in humans (Wartenberg et al., 2000). Pharmacokinetic models (Clewell et al., 2000; Bois, 2000b) were used to estimate metabolites in the kidney. Bois's uncertainty analysis (Bois, 2000b), however, revealed the presence of substantial parameter uncertainty in the kidney dose estimates (see Table 4-1, Section 4.2.2). Consequently, dose estimates for the kidney were based on equivalence of $mg/kg^{3/4}$-d instead (U.S. EPA, 1992). Rhomberg (2000) fitted empirical dose-response models to the kidney data (see Table 4-8). Following NTP guidance to combine kidney adenocarcinomas and adenomas (McConnell et al., 1986), the resulting human-equivalent LED_{01}

for kidney tumors is 33 mg/kg-d. LED_{01}s were calculated instead of LED_{10}s because kidney tumor incidences can be observed below the 10% level.

4.5.4.2. *Extrapolation to Lower Doses*

TCE-induced kidney tumors may arise through multiple modes of action (see Section 3.5.2). The preponderance of evidence suggests that TCE's GST metabolites are responsible for kidney toxicity and kidney tumors (Lash et al., 2000b). Although these metabolites have not been tested in cancer bioassays, the GST metabolite DCVC is mutagenic (Moore and Harrington-Brock, 2000). Involvement of a mutagenic metabolite would indicate use of linear extrapolation to lower doses (U.S. EPA, 1996). Cytotoxicity may also be involved, as cytotoxicity is observed in both rats and humans exposed to high levels of TCE.

Linear extrapolation from the LED_{01} of 33 mg/kg-d yields a slope factor of 3×10^{-4} per mg/kg-d ($0.01/33 = 3\times10^{-4}$).

4.5.5. Risk Estimates From Testicular Tumors in Rats

4.5.5.1. *Modeling*

Independent studies provide some evidence that TCE causes testicular tumors in rats by oral gavage and by inhalation (see Table 3-4). Health Canada derived a cancer potency estimate (a TD_{05}, the dose associated with an increased tumor risk of 5%) from the increased incidence of testicular interstitial cell tumors in Marshall rats exposed to TCE (see Table 3-4). Health Canada's dosimetry assumed cross-species equivalence of mg/kg-d. Their resulting TD_{05} was 200 mg/kg-d.

Using EPA's methodology with the same study yields an LED_{10} of 25 mg/kg-d. The eightfold ratio of Health Canada's TD_{05} to EPA's LED_{10} is attributable to a factor of 3.8 for EPA's use of 3/4-power scaling and a factor of 2.2 between the lower-bound dose at 10% response and the central-estimate dose at 5% response.

4.5.5.2. *Extrapolation to Lower Doses*

TCE-induced testicular tumors would likely result from endocrine disturbances (see Section 3.5.4). There is evidence of endocrine disturbance in both rats (Kumar et al., 2000) and humans (Chia et al., 1997; Goh et al., 1998). Endocrine disturbance in humans is one of the critical effects used in developing the RfC, where a subchronic LOAEL of 11 ppm (59 mg/m^3) was observed for decreased testosterone, decreased follicle-stimulating hormone, decreased SHBG, and increased DHEA (see Section 4.4.2). Route extrapolation yields equivalent oral doses of 0.8 mg/kg-d to 20 mg/kg-d, depending on whether TCA or DCA is used as the dose

metric (see Section 4.2.3). As a check for consistency with the tumor data, these precursor doses lie below the LED_{10} for tumors of 25 mg/kg-d.

EPA's proposed cancer guidelines (U.S. EPA, 1996) prefer to extrapolate from precursor data instead of tumor data, using an RfD or RfC for the precursor if the tumors were demonstrated to occur only subsequently to the precursor. If this is the case, then an RfD for endocrine-related tumors would be calculated by dividing the point of departure for endocrine disturbance (0.8 mg/kg-d) by the composite uncertainty factor (1,000, comprising 10 for human variation, 10 for a subchronic study, 10 for the LOAEL) previously applied to endocrine disturbance as a critical effect (see Section 4.4.3). The resulting RfD is 8×10^{-4} mg/kg-d.

4.5.6. Discussion of Alternative Cancer Risk Estimates

4.5.6.1. *Slope Factors*

Table 4-9 and Figure 4-3 compile the alternative points of departure and cancer risk estimates for TCE. Inhalation-to-oral extrapolation of estimates from the Anttila study was based on internal TCA (see Section 4.2.3), because that study quantified exposure by measuring urinary TCA. Route extrapolation is also supported by the general similarity of tumor sites from oral or inhalation exposure (see Section 3.4). Nonetheless, the best approach for route extrapolation remains a topic for further research (see Section 5).

Two sets of estimates appear to lie apart from all others. On the low end, rats appear to be less sensitive than mice or humans. On the high end, estimates from the Anttila study are rather uncertain, based on a small number of cancer cases and an assumed uniform exposure duration of 15 years. Setting aside these lowest and highest estimates, there appears to be convergence of the other estimates, even though they are derived from different sources. The remaining slope factors, per mg/kg-d, are 2×10^{-2} (derived from occupational inhalation exposure), 3×10^{-2} to 2×10^{-1} (derived from mice), and 4×10^{-1} (derived from residential drinking water exposure). Because they are supported by diverse studies and do not reflect the highest estimates (from the Anttila study) or the lowest estimates (from the rat studies), these remaining estimates constitute a middle range of risk estimates where confidence is greatest. This middle range is robust in the sense that it is not likely to be dramatically altered by a new study or by minor changes in the analysis of existing studies.

This new slope factor range, 2×10^{-2} to 4×10^{-1} per mg/kg-d, lies just above EPA's previous slope factor for TCE, 1.1×10^{-2} per mg/kg-d. The increase can be attributed principally to two factors: (1) the pharmacokinetic models predict that humans would experience higher internal doses of TCA and DCA than would mice from similar exposures (Rhomberg, 2000), perhaps due to more extensive enterohepatic recirculation (Barton and Clewell, 2000); and (2) the generation of TCA and DCA would be more efficient at the lower doses expected from environmental

exposure than from doses used in the bioassays (Rhomberg, 2000). The new slope factor range also lies just above the recent slope factor used by Cal/EPA, 1.3×10^{-2} per mg/kg-d (Cal/U.S. EPA, 1999).[71]

4.5.6.2. *Nonlinear Analyses*

Nonlinear extrapolations were attempted for liver tumors, lung tumors, and testicular tumors. For liver tumors, the point of departure was in the range of 0.5–3.1 mg/kg-d, with a dose reduction of 3,000–10,000 needed to reach a dose where there may be little concern for cancer. For lung tumors, it was problematic to determine the reduction needed to reach such a dose. For testicular tumors, an RfD of 8×10^{-4} mg/kg-d was developed.

4.5.6.3. *Joint Consideration of Slope Factors and Nonlinear Analyses*

Joint consideration of results from linear and nonlinear extrapolations is an area for which there has been no guidance. This may prove to be a complex question, as the purpose of linear extrapolation is to estimate a bound on risk whereas nonlinear extrapolation attempts to make a qualitative assurance of safety. It would not be appropriate to settle on a single result by choosing one analysis and ignoring the other.

In the meantime, linear and nonlinear analyses can be harmonized somewhat by being expressed in common units. For example, slope factors can be inverted to obtain *risk-specific doses*.[72] For an increased risk of 1 in 1 million, the slope factor range (2×10^{-2} to 4×10^{-1} per mg/kg-d) yields 2.5×10^{-6} to 5×10^{-5} mg/kg-d as a range of risk-specific doses. Similarly, for a risk of 1 in 10,000, the range is 2.5×10^{-4} to 5×10^{-3} mg/kg-d. Nonlinear extrapolation can be expressed as a dose where there may be little concern for cancer. For example, for a point of departure of 1.8 mg/kg-d (the midpoint of 0.5–3.1 mg/kg-d, the point-of-departure range for liver tumors) and a dose reduction of 6500 (the midpoint of 3000–10,000, the dose-reduction range developed for mouse liver tumors), a dose where there may be little concern for liver cancer is roughly 3×10^{-4} mg/kg-d ($1.8/6500=3\times10^{-4}$). Higher or lower results could be obtained by using other choices from these two ranges, but the midpoints serve to illustrate the comparison between linear and nonlinear health benchmarks.

Figure 4-3 shows that for TCE the health benchmarks under nonlinear extrapolation are approximately 10–100 times the doses associated with an increased cancer risk of 1 in 1 million

[71]Cal/EPA's cancer estimates are based on liver tumors in mice (NCI, 1976; Maltoni et al., 1986), modeled to an LED_{10} of 7.9 mg/kg-d using total amount of TCE metabolized as the dose metric. Using linear extrapolation to lower doses, their slope factor is $0.10/LED_{10} = 1.3\times10^{-2}$. Cal/EPA considered nonlinear extrapolation to be insufficiently supported by the data.

[72]A *risk-specific dose* is calculated by dividing a risk level by a slope factor. For example, if the slope factor is 2 per mg/kg-d, then the risk-specific dose associated with a risk of 1 in 1 million is $10^{-6}/(2 \text{ per mg/kg-d}) = 5\times10^{-7}$ mg/kg-d.

under linear extrapolation. In other words, nonlinear extrapolation leads to doses that would be associated with increased cancer risks of 1 in 100,000 to 1 in 10,000 under linear extrapolation. This suggests that if confidence increases in the appropriateness of nonlinear extrapolation for TCE, then levels that we now associate with risks approaching 1 in 10,000 may be considered virtually safe in the future.

4.5.6.4. *Sensitive Populations and Cumulative Risks*

Information is beginning to emerge about differences in metabolism, disease, and other factors that make humans vary in their response to TCE, as well as the potential for other chemicals to alter TCE's metabolism and toxicity. This information indicates that a single risk value is not appropriate to describe the differential effects of TCE. For this reason, the alternative slope factors have not been consolidated into a single estimate. Rather, a range of slope factors has been described. Depending on the characteristics of the exposed population and the exposure scenario, each risk assessment should select an appropriate slope factor from this range. Risk assessments involving the presence of risk factors such as diabetes or alcohol consumption, or high background exposure to TCE or its metabolites, would more appropriately choose a higher slope factor. Conversely, lower slope factors may be appropriate when these risk factors and background exposures are not involved.

It will be a challenge for future research to quantify the differential risk indicated by each risk factor or exposure scenario (see Section 5). In this way, nonlinear cancer assessments will stop resembling single-point RfDs and will move toward risk ranges with the flexibility to quantitatively accommodate population-specific and site-specific considerations.

4.5.7. Sources of Uncertainty in the Dose-Response Assessment

All risk assessments involve uncertainty, as study data are extrapolated to make inferences about potential effects from human environmental exposure. One type of uncertainty stems from different interpretations of the available data (known as model uncertainty), another from estimation errors due to incomplete or imprecise data (parameter uncertainty). An understanding of uncertainty can be used:

- To make more informed choices during the conduct of a risk assessment, and
- To better characterize the range of plausible risk estimates for different populations.

4.5.7.1. *Model Uncertainty*

This assessment's exploration of multiple modeling approaches affords an opportunity to understand and address different sources of model uncertainty. The full extent of model

uncertainty cannot be quantified, only the models that have been analyzed. In this assessment, several sources of model uncertainty have been discussed:

- Two pharmacokinetic models initially led to risk estimates that differed by 15-fold (see Table 4-4). To reduce this uncertainty, the models were fitted to additional data sets (Bois, 2000a,b). This calibration improved the models and made the models' results more compatible, reducing this source of model uncertainty (see Section 4.2.1).
- A mechanism-based model assumption that TCE-induced liver tumor incidences are proportional to a species' background liver tumor incidence results in 100-fold lower human risk estimates (see Section 4.5.2). Because current mechanistic understanding suggests that cross-species extrapolation is far more complex (see Section 3.5.1), this assessment chose estimates not based on this assumption.
- Different exposure routes yield different proportions of metabolite formation. Depending on whether TCA formation or DCA formation is used as the basis of comparison, route extrapolations can differ by 25-fold (see Section 4.2.3). To convey an appreciation for this unresolved uncertainty, this assessment has developed estimates using both approaches.
- Different exposure patterns[73] may yield different risks, but this has not been well investigated. In the absence of TCE-specific data, this assessment has followed the common, neutral practice of assuming that equal risks result from equal cumulative doses. There are some data, however, to suggest that this approach may underestimate risks from short-duration exposures (see Section 4.5.2).
- The choice between linear and nonlinear extrapolation to environmental doses creates two distinct classes of estimates (see Sections 4.5.2, 4.5.3). This dichotomous uncertainty stems from different interpretations of the information on TCE's mode-of-action (see Section 3.5). Some toxicologists maintain that cross-species differences would make humans less sensitive to TCE according to the modes of action proposed for mice and rats, suggesting less concern for human cancer. Other scientists maintain that such conclusions are premature without more specific definition and experimental confirmation of the active agents and sequences of key events leading to cancer. In addition, some of TCE's effects on cell signaling may augment ongoing processes and conditions in some groups of humans, leading to an increased cancer risk for some. EPA's proposed cancer guidelines take the latter view, that the active agents and key events be identified in order to establish the mode-of-action. Consequently, this

[73]*Exposure patterns* refer to magnitude, frequency, duration, and timing of exposure. For example, exposure to 30 ppm for 10 hr at age 30 and exposure to 300 ppm for 1 hr at age 1 are two different exposure patterns with a total exposure of 300 ppm-hr.

assessment has prepared estimates using both linear and nonlinear approaches, the former to bound the risks and the latter to demonstrate the degree of uncertainty and value of further mechanistic research. In addition, this assessment has made efforts to develop risk estimates from human studies as an alternative to extrapolating from animal models.

4.5.7.2. *Parameter Uncertainty*

Uncertainty analyses and confidence intervals were developed for some of the key parameters used in this assessment. Each description of parameter uncertainty assumes that the underlying model is valid.

- Uncertainty in the pharmacokinetic data is reflected by 95% confidence intervals spanning approximately 100-fold for the liver, 14,000-fold for the lung, and 5,000-fold for the kidney (see Table 4-1, Section 4.2.2). Because of this high uncertainty for lung and kidney, this assessment reverted to default animal-to-human dose-scaling models for these organs. In all cases, median dose estimates were used in subsequent calculations.
- Uncertainty in the animal dose-response data is reflected by the ratio of ED10s to LED10s. These generally do not exceed a factor of 2 (Rhomberg, 2000).

Table 4-1. Distributions of potential dose metrics from the uncertainty analysis of the pharmacokinetic models

		Ratio of dose estimate at each percentile to the median[a]							
Dose metric	**GSD**	**1**	**2.5**	**10**	**50**	**90**	**97.5**	**99**	**Span of 95% CI[b]**
Mouse liver TCA AUC	3.2	1/15	1/10	1/4	1	4	10	15	100
Mouse liver DCA AUC	3.6	1/20	1/12	1/5	1	5	12	20	144
Mouse lung CH AUC	11.7	1/300	1/120	1/23	1	23	120	300	14,400
Mouse lung CH max	12.5	1/360	1/140	1/25	1	25	140	360	19,600
Rat kidney thiol	9.0	1/170	1/74	1/17	1	17	74	170	5,476

[a]Assuming lognormally distributed errors.
[b]Ratio of estimate at 97.5 percentile to estimate at 2.5 percentile.
Source: Adapted from Bois, 2000b, Table 5–7; Rhomberg, 2000, Table 15.

Table 4-2. Oral studies supporting development of an RfD

Study	Species	Duration; exposure route	Exoerimental doses (mg/kg/day)[1,2]	Organ; effect	Human-equivalent dose[2,3]
Tucker et al. (1982) and Sanders et al. (1982)	Mouse	Subchronic (6 months) Oral - drinking water	0, 18[4], 217[5], 393, 660 (m) 0, 18[4], 193[5], 437, 793 (f)	Liver: liver weight-body weight ratio Immune system: antibody (plaque)-forming cell assay	NOAEL, PK - adj. 1 mg/kg-d NOAEL 29 mg/kg-d
Buben and O'Flaherty (1985)	Mouse	Subchronic (6 weeks) Oral - gavage	0, 100, 200, 400, 800, 1600, 2400, 3200	Liver: liver weight-body weight ratio	LOAEL, PK - adj. 1 mg/kg-d
Berman et al. (1995) and Moser et al. (1995)	Rat	14 days Oral - gavage	0, 50[6], 150[7], 500, 1500	Liver: liver weight-body weight ratio Neurobehavioral	LOAEL, PK - adj. 0.3 mg/kg-d BMD_{10}, PK - adj. (Weibull) 0.6 mg/kg-d (Power) 0.5 mg/kg-d NOAEL 40 mg/kg-d
Maltoni et al. (1986)	Rat	Chronic bioassay (52 weeks exposure, followed until natural death) Oral - gavage	0, 50, 250	Kidney: renal megalonucleocytosis	NOAEL 10 mg/kg-d
Dawson et al. (1993)	Rat	Developmental Oral - drinking water	0, 1.5[8], 1100 ppm • 0.18, 129 mg/kg/day	Developmental: cardiac anomalies	LOAEL 34 mg/kg-d NOAEL 0.05 mg/kg-d
Narotsky et al. (1995)	Rat	Developmental Oral - gavage	0, 10, 32, 101, 320, 475, 633, 844, 1,125	Developmental: anopthalmia/ micropthalmia	NOAEL 9 mg/kg-d BMD_{10} 133 mg/kg-d
Griffin et al. (2000c)	Mouse	Subchronic (4 weeks and 32 weeks) Oral - drinking water	0, 21, 100, 400	Immune system/liver: serum antinuclear antibodies (ANA), histopathologcial changes, serumalanine aminotransferase	LOAEL 4 mg/kg-d

[1] NOAELs or LOAELs are underlined.
[2] From Barton and Clewell (1999).
[3] Human equivalent doses calculated using RfD methodology (Barnes and Dourson, 1988) and scaling to the ratio of body weights to the 0.75 power.
[4] Tucker et al. (1982).
[5] Saunder et *al. (1982)*.
[6] Berman et al. (1995).
[7] Moser et al. (1995).
[8] NOAEL.

Table 4-3. Inhalation studies supporting development of an RfC

Study	Species	Duration; exposure route	Concentration[1]	Organ; effect	Human-equivalent concentration[2,3]
Kjellstrand et al. (1983)	Mouse	Subchronic (30 days) inhalation	0, 37, 75, 150, 300 ppm	Liver: liver weight-body weight ratio	LOAEL,PK-adj. 12 ppm BMD_{10}, PK - adj. (f) 5 ppm (m) 9, 11 ppm
Chia et al. (1997) Goh et al. (1998) Chia et al. (1996)	Human	Subchronic (mean =5 years) inhalation	30 ppm (mean)	Endocrine/Liver: FSH, testosterone, DHEAS[4], FAI[4], SHBG[4], insulin	LOAEL 11 ppm
Maltoni et al. (1986)	Rat	Chronic bioassay (104 weeks) inhalation	0, 100, 300, 600 ppm	Kidney: megalonucleocytosis	NOAEL, PK - adj. 21 ppm
Ruijten et al. (1991)	Human	Subchronic (mean =16 years) inhalation	44 ppm (calculated from mean cumulative exposure)	CNS: massiter reflex latency	LOAEL 16 ppm
Rasmussen et al. (1993)	Human	Subchronic (mean = 7 years) inhalation	40 - 60 mg/l urinary TCA - • 20 ppm[5]	CNS: motor dyscoordination	LOAEL 7 ppm
Vandervort and Pelakoff (1973)	Human	Subchronic (8 years) inhalation	32 ppm	CNS: drowiness, fatigue, dizziness, eye irritation	LOAEL 11 ppm
Okawa and Bodner (1973)	Human	Subchronic inhalation	71 mg/l urinary TCA (average) - •20 - 30 ppm[5]	CNS: nausea, headache, dizziness, fatigue, upper respiratory irritation	LOAEL 7-11 ppm
Arito et al. (1994)	Rat	Subchronic (6 weeks) inhalation	0, 50, 100, 600 ppm	CNS: heart rate, electroencephalographic changes	LOAEL,PK- adj. 9 ppm BMD_{10}, PK - adj. 5 ppm (wakefulness)
Kumar et al. (2000)	Rat	Subchronic (12 weeks and 24 weeks) inhalation	0, 376 ppm	Endocrine/Reproductive: testosterone, testicular cholesterol, 17 B-hydroky steroid dehydrogenase, glucose 6-P- dehydrogenase, sperm number and motility	LOAEL 41 ppm

[1] NOAELs or LOAELs are underlined.

[2] Human equivalent doses calculated using RfC methodology (U.S. EPA, 1994) for a category 3 gas, extrathoracic effects.
$NOAEL^*_{[HEC]}$ (mg/m^3) = $NOAEL^*_{[ADJ]}$ (mg/m^3) x $[(H_{b/g})_A / (H_{b/g})_H]$.
The value of 1.0 is used if $(H_{b/g})_A > (H_{b/g})_H$. $(H_{b/g})_A$ = 15.9, mouse (Fisher, 2000); 14, mouse, 18.5, rat (Clewell et al., 2000). $(H_{b/g})_H$ = 9.13, male, 11.15, female (Fisher, 2000); 9.2 (Clewell et al., 2000).

[3] HECs for mice and rats are from Barton and Clewell (1999).

[4] DHEAS - dihydroepiandrosterone sulphate, FAI - free androgen index, SHBG - sex hormone binding globin.

[5] U-TCA interpolated to atmospheric concentration assuming 50 mg/l U-TCA is equivalent to 20 ppm (Axelson et al., 1994).

Table 4-4. Human-equivalent LED_{10}s (mg/kg-d) derived from liver tumor incidences in four mouse studies (NTP males or females, NCI males or females), using two dose metrics (TCA area-under-the-curve, DCA area-under-the-curve) and four pharmacokinetic models (Fisher 2000; Bois, 2000a; Clewell et al., 2000; Bois, 2000b)

	Based on TCA area-under-the-curve				Based on DCA area-under-the-curve			
	Fisher	Bois/ Fisher	Clewell	Bois/ Clewell	Fisher	Bois/ Fisher	Clewell	Bois/ Clewell
Male mice (NTP)	0.19	1.47	0.077	0.23	0.77	NA	12.05	1.15
Female mice (NTP)	0.33	3.03	0.15	0.45	1.39	NA	24.39	2.33
Male mice (NCI)	0.83	4.76	0.24	0.83	3.45	NA	40.00	4.35
Female mice (NCI)	2.63	18.52	1.01	3.57	10.75	NA	169.49	18.18
Mean[1] of 4 exp'ts	0.40	3.14	0.16	0.50	1.67	NA	25.82	2.52

NA = not available.

[1]Harmonic mean = $N/(\sum 1/X_i)$; equivalent to averaging slope factors (which are inversely proportional to LED_{10}s), then taking the reciprocal to convert back to a dose.

Source: Adapted from Rhomberg (2000) (LED_{10}s are computed as 0.10 divided by slope factors in Rhomberg, 2000, Table 4).

Table 4-5. Extending the observed range with a study of less-than-lifetime dosing of TCE

Concentration (ppm)	Duration (wk)	Exposure index (conc × dur)	Hepatoma incidence
0	8	0	1/41 (2%)
100	8	800	3/36 (8%)
600	8	4,800	4/23 (17%)
0	78	0	4/66 (6%)
100	78	7,800	2/53 (4%)
300	78	23,400	8/59 (14%)
600	78	46,800	13/61 (21%)

Source: Adapted from Maltoni et al., 1986.

Table 4-6. Extending the observed range with studies of TCE's metabolites

Compound	Dose (DCA AUC)	Liver tumor incidence
DCA	1.38	3/21 (14%)
	14.01	1/18 (5%)
	63.66	8/12 (67%)
	88.4	25/30 (83%)
TCA	0.07	2/9 (22%)
	7.12	8/21 (38%)
	40.8	21/24 (88%)
TCE	67	(61%)
	71	(86%)
	67	(65%)

Source: Adapted from Chen, 2000.

Table 4-7. Human-equivalent LEC_{10}s (ug/m^3) derived from lung tumor incidences in four mouse studies, using RfC dosimetry

	LEC_{10}
Female ICR mice (Fukuda)	1.4×10^5
Male Swiss mice (Maltoni)	1.3×10^5
Female Swiss mice (Maltoni)	3.6×10^5
Male B6C3F1 mice (Maltoni)	2.8×10^5
Mean of 4 experiments	1.9×10^5

Source: Adapted from Rhomberg, 2000. (LEC_{10}s are computed as 0.10 divided by unit risks in Rhomberg, 2000, Table 9).

Table 4-8. Human-equivalent LED_{01}s (mg/kg-d) derived from kidney tumor incidences in rat studies, using 3/4-power scaling

	Based on cross-species scaling factor of 3/4-power of relative body weight
Male and female rats, 5 strains pooled (NTP), gavage, adenocarcinomas only	6.7×10^{1}
Male and female rats, 5 strains pooled (NTP), gavage, adenocarcinomas and adenomas[1]	3.3×10^{1}
Male Sprague-Dawley rats (Maltoni), inhalation, adenocarcinomas only	N/A

[1]NTP guidance (McConnell et al., 1986) recommends combining adenocarcinomas and adenomas; Rhomberg (2000) Table 12 states that including adenomas yields values about half those for adenocarcinomas alone.
Source: Adapted from Rhomberg, 2000. (LED_{01}s are computed as 0.01 divided by slope factors in Rhomberg, 2000, Table 13.)

Table 4-9. Compilation of cancer estimates

	Point of departure (mg/kg-d)	Slope factor $(mg/kg\text{-}d)^{-1}$	Risk-specific dose[a] (mg/kg-d)
Cancer estimates based on human studies			
Liver cancer			
Finnish cohort[b]	1.4[c]	7×10^{-2}	1.4×10^{-5}
Kidney cancer			
Finnish cohort[b]	0.05[c]	2×10^{0}	5×10^{-7}
German cohort	5[c]	2×10^{-2}	5×10^{-5}
Non-Hodgkin's lymphoma			
Finnish cohort[b]	0.014[c]	7×10^{0}	1.4×10^{-7}
New Jersey cohort	0.25[c]	4×10^{-1}	2.5×10^{-6}
Cancer estimates based on mouse studies			
Liver cancer			
Mechanism-based model[d]	Not applicable	8×10^{-4}	1.25×10^{-3}
Mechanism-based model[e]	Not applicable	8×10^{-2}	1.25×10^{-5}
Linear extrapolation	0.5–3.1	3×10^{-2}–2×10^{-1}	$0.5–3.1\times10^{-5}$
Nonlinear extrapolation	0.5–3.1	Not applicable	(3×10^{-4})[f]
Lung cancer[g]	1.7–4.8	Not applicable	(Not calculable)[f]
Cancer estimates based on rat studies			
Kidney cancer	33[h]	3×10^{-4}	3.3×10^{-3}
Testicular cancer	25	Not indicated	(8×10^{-4})[f]

[a]Dose associated with an upper-bound increased cancer risk of 1 in 1 million, calculated as 10^{-6}/slope factor. When nonlinear low-dose extrapolation is used, this is a dose considered to pose minimal cancer risks, calculated as the point of departure divided by the composite risk reduction factor from a nonlinear dose-response analysis.

[b]For the Finnish cohort, the slope factors are based on route extrapolation from air unit risks, using a correspondence between inhaled and oral doses giving equal TCA area-under-the-curve (75 mg/m^3 ~ 1 mg/kg-d, see Section 5.2.3). Air unit risks for liver cancer, kidney cancer, and non-Hodgkin's lymphoma are 9×10^{-7}, 3×10^{-5}, and 9×10^{-5} per $\mu g/m^3$, respectively.

[c]The point of departure (an LED_{10}) is calculated from the slope factor (LED_{10}=0.10/slope factor).

[d]From a mechanism-based model, assuming that tumor response is proportional to background liver tumor incidence (Chen, 2000).

[e]From a mechanism-based model (see note m), without assuming that liver tumor response is proportional to background liver tumor incidence.

[f]This is a dose considered to pose minimal cancer risks, calculated as the point of departure divided by the composite risk reduction factor from a nonlinear dose-response analysis. This is not a dose associated with a 1-in-1 million cancer risk.

[g]The point of departure is based on route extrapolation from the point of departure for an inhalation study in mice using a correspondence between inhaled and oral doses giving equal TCA area-under-the-curve [75 mg/m^3 ~ 1 mg/kg-d, see Section 5.2.3]. The point of departure for mouse lung tumors (an LEC_{10}) spans a range of 1.3×10^5 to 3.6×10^5 $\mu g/m^3$.

[h]The point of departure is an LED_{01} rather than an LED_{10} (see Section 5.5.3).

Figure 4-1. Extending the observed range with less-than-lifetime dosing

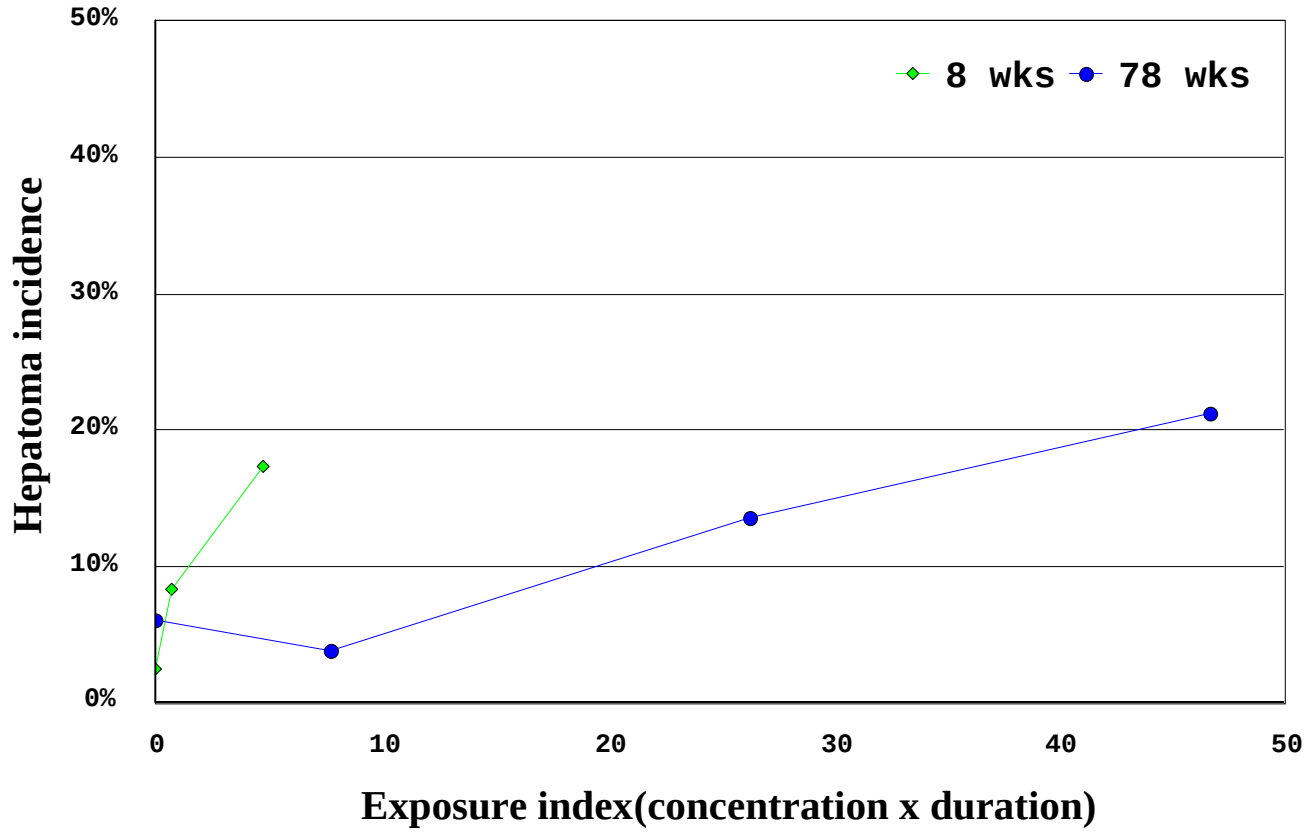


Figure 4-2. Extending the observed range with studies of TCE's metabolite

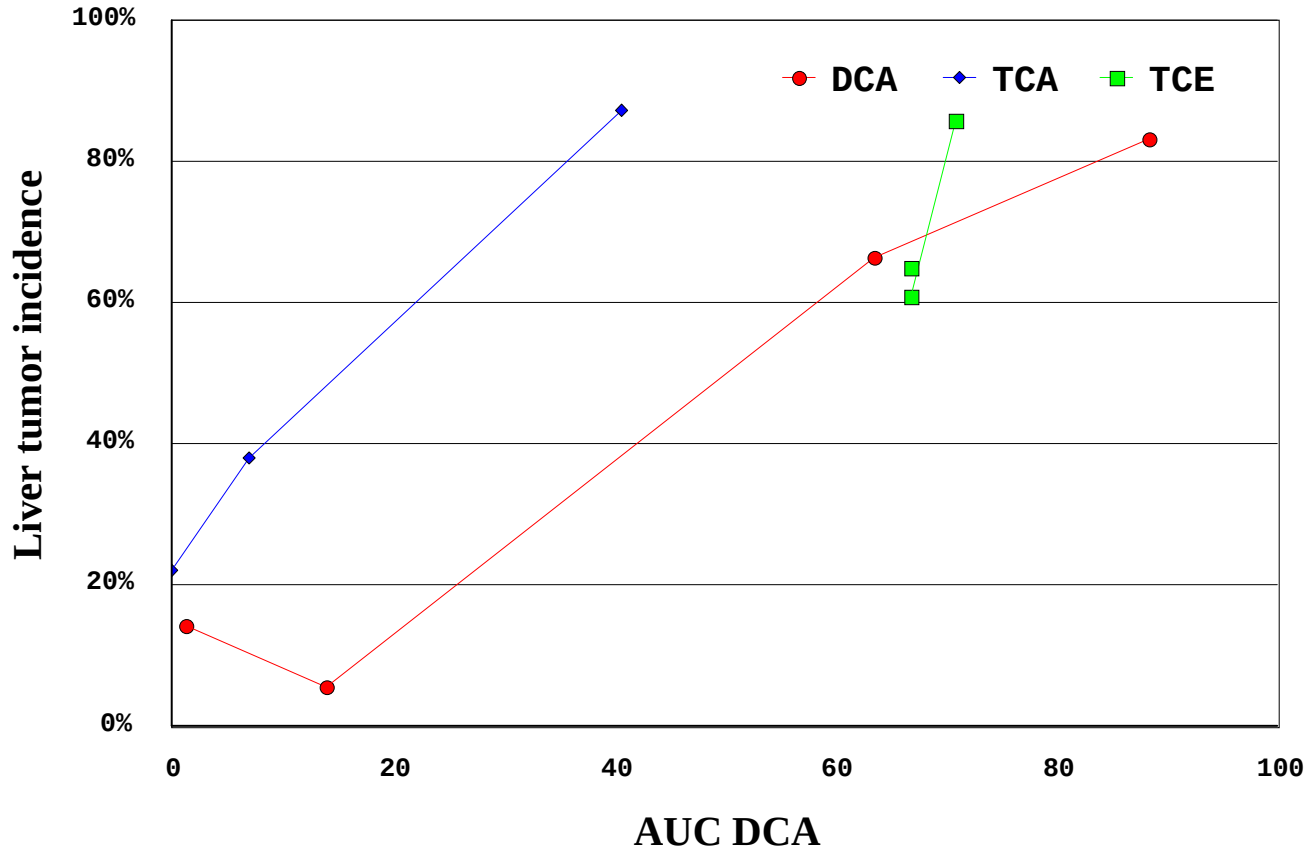
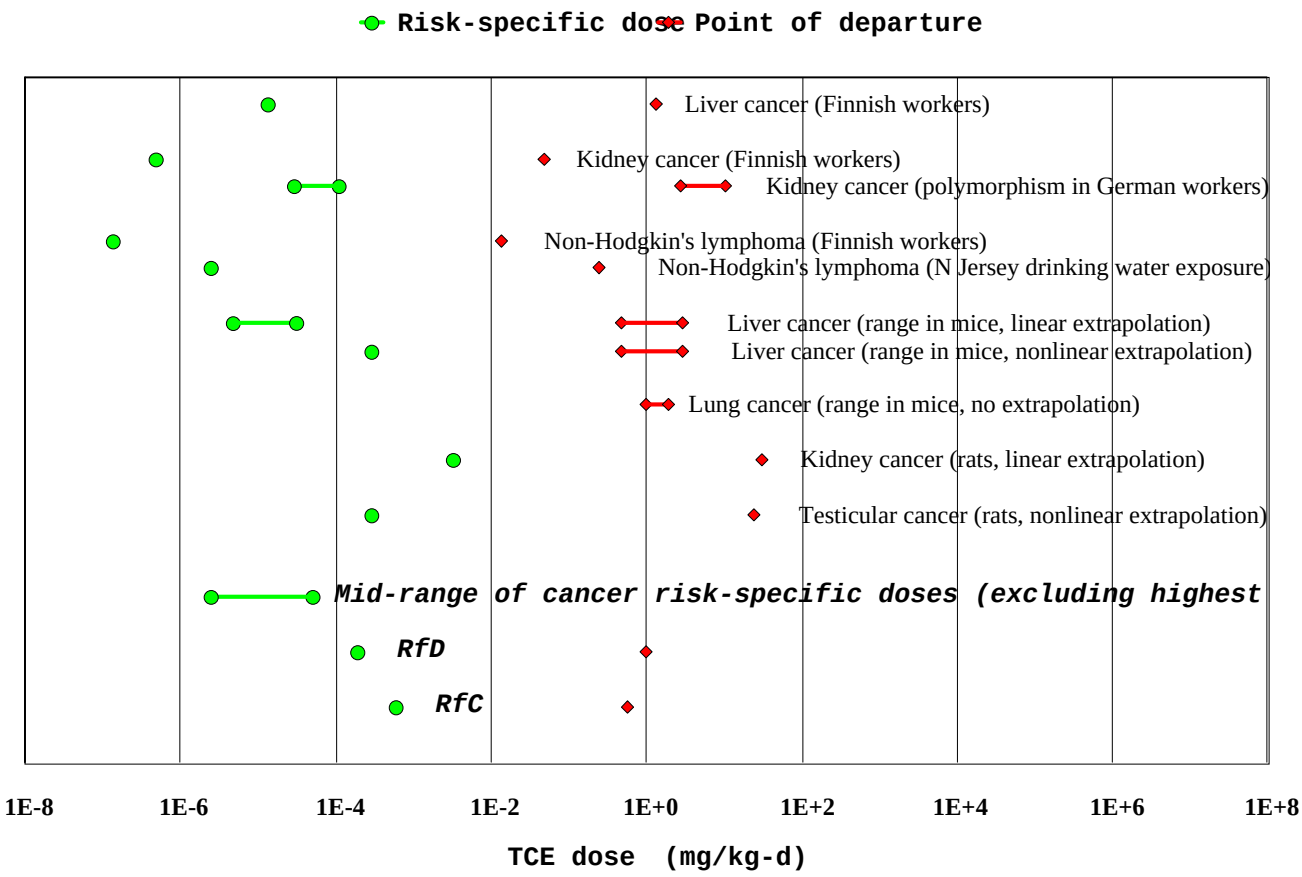


Figure 4-3. TCE health benchmarks



5. RESEARCH NEEDS

Although the database for TCE is rather extensive and much has been learned in recent years, this is a time when new research could significantly increase understanding and lead to later refinement of this health risk assessment. Many research needs were discussed in the state-of-the-science papers that comprise volume 1 of this assessment, and this research promises to increase understanding of TCE's adverse health effects, the active agents and modes of action for each effect, and their relevance to human environmental exposure. This section focuses on those research needs that left this assessment with open questions that were filled by default assumptions and methods. Further research into these areas could, therefore, have direct bearing on specific sections of this assessment. The practical importance of describing these research needs is not to decrease confidence in this assessment, as all assessments have areas that can be improved by further research, but to be explicit about what specific research could help resolve the major open questions in this assessment.

5.1. RESEARCH NEEDS FOR HAZARD ASSESSMENT

(a) TCE's effects other than cancer have not been adequately identified or studied. There is a lack of definitive chronic studies of effects other than cancer (see Section 3.4). Investigation into the modes of action for effects other than cancer lags behind what has been developed for TCE-induced cancer (see Section 3.5).

(b) With EPA's proposed cancer guidelines becoming more specific (U.S. EPA, 1999), it is now apparent that what is needed is a description of the sequence of key events for each tumor type and the dose levels at which those events begin to occur. Although there has been much progress in developing and investigating several plausible modes of action, the active agents and key events have not yet been identified (see Section 3.5).

(c) For TCE-induced liver cancer, further research is needed to identify the key events and the doses at which they begin to occur. Characterizing biological markers of the liver tumors from the TCE bioassays would promote understanding of the respective roles and interaction of TCA and DCA in the TCE-induced tumors (see Section 3.5.1).

(d) Fuller identification of the mode-of-action and associated key events for kidney tumors, especially with respect to the potential role of VHL mutations in human kidney cancer, could affect the description of TCE as either "carcinogenic to humans" or "likely to be carcinogenic to humans." Replication of recent findings in another cohort showing kidney tumors could warrant a future description of TCE as "carcinogenic to humans" (see Section 1.3).

5.2. RESEARCH NEEDS FOR DOSE-RESPONSE ASSESSMENT

(a) Route extrapolations can differ by 25-fold, depending on whether internal TCA or DCA is used as the dose metric. Further research could identify the appropriate internal dose metric for each toxic effect (see Section 4.2.3).

(b) There is a need for consensus on a preferred empirical model to be used for each type of data set. This assessment's experience with benchmark dose modeling revealed differences of more than an order of magnitude depending on which empirical model was used. This invites "model shopping" and introduces too much uncertainty for use in risk assessments that support public health decisions (see Section 4.3.2, Table 4-2).

(c) The RfD includes a data-derived factor of 50 for human variation and default factors of 100 overall for uncertainty in extrapolating from animals to humans, from subchronic studies to lifetime exposure, from effect levels to NOAELs, and from single-chemical toxicity tests to complex exposures involving multiple chemicals. This latter 100-fold factor indicates the potential for future research to reduce uncertainty and improve the assessment's accuracy (see Section 4.3.3. 4.3.4).

(d) There is considerable pharmacokinetic and pharmacodynamic uncertainty for children. An approach is needed for evaluating the need for and size of an additional factor to ensure that the RfD and RfC are protective of children, while also providing incentives for research to quantify the potential difference in sensitivity between children and adults.

(e) At low doses, the mode-of-action for liver tumors may be nonlinear; nonetheless, current data do not identify how far below the LED_{10}s sublinearity begins to prevail. Further research is needed to identify the key precursor events and describe their dose-response curves (see Section 4.5.2).

(f) The mode-of-action for lung tumors is undeveloped with respect to developing dose-response curves for the key events. In addition, there is substantial parameter uncertainty in current pharmacokinetic estimates of a dose metric in the lung (see Section 4.5.3).

(g) Similarly, there is substantial parameter uncertainty in current pharmacokinetic estimates of a dose metric in the kidney (see Section 4.5.4).

(h) This assessment identifies several risk factors that would make a population more sensitive. Further research is needed to estimate the magnitude of the increased risk. Understanding the functional relationship between a risk factor and the associated disease is a critical research need that presently prevents estimating the differential risk faced by sensitive populations (see Sections 2.4, 2.5, 2.6, 4.5.6).

(i) It may be important to understand the joint effect of several risk factors together. If the effects of several factors are multiplicative, the combined effect could be quite large (see Section 4.5.6).

(j) Risk assessment guidelines and methods will need to explicitly consider the effect of background exposures as risk assessments consider the cumulative effect of multiple chemicals and stressors on an exposed population. Finding a safe dose in an otherwise unexposed population does not mean that that dose is safe when background exposures are considered (see Section 1.6).

5.3. RESEARCH NEEDS FOR EXPOSURE ASSESSMENT

(a) Limited data indicate that TCE has been detected in all of eight samples of human milk from four U.S. urban areas. Quantification of levels of TCE and its metabolites in milk, in both highly exposed and relatively unexposed populations, is a critical research need that presently prevents comparing levels in milk with levels allowed in drinking water (see Section 1.5).

(b) As TCE's metabolites have a large role in TCE-induced toxicity, there is a need to estimate cumulative exposure to these metabolites from all parent compounds, sources, and pathways (see Section 1.6).

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