Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues

Committee on Human Health Risks of Trichloroethylene, National Research Council

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Key Scientific Issues

Committee on Human Health Risks of Trichloroethylene

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

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Preface

Trichloroethylene, an environmental contaminant, is widespread because of its extensive use as a degreasing agent, because of its use as a chemical intermediate in a variety of industries, and because of disposal practices. To help protect the public from potential health effects caused by exposure to trichloroethylene, government and state agencies perform risk assessments to develop guidelines intended to restrict the public's contact with the chemical. Such risk assessments require consideration of a wealth of scientific information on trichloroethylene. Government agencies and the scientific community have engaged in much debate over the quality of some data and how to assess the information. Because several government agencies share responsibility for cleaning up contaminated sites, an interagency group composed of the U.S. Department of Defense, the Department of Energy, the Environmental Protection Agency, and the National Aeronautics and Space Administration requested a study by the National Research Council (NRC) to provide independent guidance on scientific issues to support an objective and scientifically balanced health risk assessment for trichloroethylene.

In response to the agencies' request, the NRC convened the Committee on Human Health Risks of Trichloroethylene, which prepared this report. The members of the committee were selected for their expertise in pharmacokinetics, kidney toxicology, liver toxicology, reproductive and developmental toxicology, neurotoxicology, inhalation toxicology, immunotoxicology, carcinogenesis, epidemiology, physiologically based pharmacokinetic modeling, biostatistics, and risk assessment. Biographical information on the committee members is provided in Appendix A.

This report presents the committee's assessment of the critical scientific issues that should be addressed in any health risk assessment of trichloroethylene. The guidance is intended to help agencies characterize the hazards from trichloroethylene. The committee also provides guidance on the development of physiologically based pharmacokinetic modeling, dose-response assessments, and other factors to consider in performing quantitative risk assessments of cancer and non-cancer risks from trichloroethylene.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following

individuals for their review of this report: Adnan Elfarra, University of Wisconsin at Madison; Jeffrey Fisher, University of Georgia; Poh-Gek Forkert, Queen's University; James Gnarra, Louisiana State University School of Medicine; David Hoel, Medical University of South Carolina; James Klaunig, Indiana University School of Medicine; Jeffrey Larson, Tanox, Inc.; Richard Miller, University of Rochester; K. Michael Pollard, The Scripps Research Institute; Martha Sandy, California Environmental Protection Agency; and William Valentine, Vanderbilt University.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Sam Kacew, University of Ottawa, and John C. Bailar, University of Chicago. Appointed by the NRC, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

The committee gratefully acknowledges the individuals who made presentations to the committee at its public meetings. A list of those individuals is provided in Appendix B. The committee also thanks Richard Canady, who was with the U.S. Office of Science and Technology Policy during the first half of the study, for coordinating the committee's interactions with the interagency sponsors, facilitating responses to data requests, and providing background information.

The committee is grateful for the assistance of NRC staff in preparing the report. It particularly wishes to acknowledge the outstanding support from project director Susan Martel, who coordinated the project and contributed to the committee's report. Other staff members who contributed to this effort are James Reisa, director of the Board on Environmental Studies and Toxicology; Mirsada Karalic-Loncarevic, research associate; and Tamara Dawson, senior program assistant.

Finally, I would like to thank all the members of the committee for their efforts throughout the development of this report.

Rogene Henderson, Ph.D.
Chair, Committee on Human Health Risks of Trichloroethylene

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Summary

Trichloroethylene is a solvent used widely as a degreasing agent. It is a common environmental contaminant at Superfund sites and at many industry and government facilities, including certain manufacturing operations (e.g., aircraft, spacecraft). Releases to air occur primarily from degreasing operations. Trichloroethylene is also found in soils and surface water as a result of direct discharges and in groundwater due to leaching from disposal operations. Indoor air can become contaminated because of volatilization from contaminated water supplies and use of certain consumer products. Vapor intrusion through walls and floors can be a source of indoor exposure in buildings near contaminated groundwater.

To help protect the public from potential health effects caused by exposure to trichloroethylene, government agencies conduct risk assessments to develop exposure guidelines intended to restrict human contact with the chemical. This requires consideration of a great deal of scientific information on trichloroethylene. There has been much debate about the quality of some sources of information and how to assess the collective evidence. Because several government agencies share responsibility for cleaning contaminated sites, an interagency group composed of the U.S. Department of Defense, Department of Energy, Environmental Protection Agency (EPA), and the National Aeronautics and Space Administration requested that the National Research Council (NRC) provide independent guidance on scientific issues related to trichloroethylene. In response to this request, the NRC convened the Committee on Human Health Risks of Trichloroethylene, which prepared this report.

THE COMMITTEE'S TASK AND APPROACH

The committee was asked to examine issues critical to developing an objective, realistic, scientifically based health risk assessment for trichloroethylene. It was asked to focus on hazard characterization and mode of action for trichloroethylene toxicity; possible approaches to synthesize epidemiologic data for characterization of hazard; human susceptibility in different subpopulations or life stages; evidence for effects from exposure to trichloroethylene alone compared with that for effects from mixtures of chemicals that include trichloroethylene; physiologically based pharmacokinetic (PBPK) modeling; dose-response assessment; and issues related to quantitative assessment of cancer and non-cancer risks. Special attention was given to the availability of appropriate data and methods to implement the committee's recommendations as well as the distinction between data analysis and data generation. The committee was asked

to distinguish between issues that can be addressed through short-term analyses and issues that are more appropriately addressed through medium- or long-term research projects. The committee was not asked to perform a risk assessment or to address risk management issues.

To accomplish its task, the committee held public data-gathering sessions to hear from the sponsoring agencies, other invited speakers, representatives from citizens' groups, and the public. The committee reviewed a large body of technical material on trichloroethylene, including relevant scientific literature, a draft risk assessment by EPA released in 2001, scientific and technical review comments on that draft assessment, and additional information provided by the sponsoring agencies and other interested parties. Because of the extent of the scientific literature on trichloroethylene, the committee took advantage of recent compilations of information as starting points and evaluated new literature to assess how the state of knowledge has advanced.

In this report, the committee provides guidance in three major categories: hazard characterization, PBPK modeling, and dose-response assessment. The section on hazard characterization provides guidance for identifying and characterizing risks to human health. Intrinsic and extrinsic factors that could modify those risks are discussed, and attention is given to issues related to susceptibility and to mixtures containing trichloroethylene. PBPK models are reviewed, and dose-response issues related to the database on trichloroethylene are considered.

THE COMMITTEE'S EVALUATION

The committee found that the evidence on carcinogenic risk and other health hazards from exposure to trichloroethylene has strengthened since 2001. Hundreds of waste sites in the United States are contaminated with trichloroethylene, and it is well documented that individuals in many communities are exposed to the chemical, with associated health risks. Thus, the committee recommends that federal agencies finalize their risk assessment with currently available data so that risk management decisions can be made expeditiously.

Hazard Characterization

Synthesizing Epidemiologic Data

A large body of epidemiologic data is available on trichloroethylene and possible cancer outcomes, and quantitative analysis of the collective evidence will be the most informative for characterizing cancer hazards. Two approaches that used meta-analytic techniques were developed by Wartenberg et al. (2000),[1] whose analysis EPA used in its draft health risk assessment, and Kelsh et al. (2005),[2] who developed their analysis after EPA's assessment. The committee found several weaknesses in the techniques used in both analyses. Problems included the use of a tiered system to classify and weigh studies, separate analyses of case-control and

[1] Wartenberg, D., D. Reyner, and C.S. Scott. 2000. Trichloroethylene and cancer: Epidemiologic evidence. Environ. Health Perspect. 108(Suppl. 2):161-176.

[2] Kelsh, M.A., M. Weingart, J. Mandel, P. Mink, D. Alexander, R. Basu, R. Kalmes, and M. Goodman. 2005. A Meta-Analysis of Epidemiology Studies of Occupational TCE Exposure and Selected Cancers. Presentation by M.A. Kelsh at the Third Meeting on Assessing Human Health Risks of Trichloroethylene, June 9, 2005, Irvine, CA.

cohort studies, and the fact that these analyses did not consider identifying amounts of exposure in the studies. Another problem was the subjective assessment of quality to exclude or categorize studies. For example, Wartenberg et al. classified one study that showed a strong positive association between trichloroethylene and kidney cancer as being of the highest quality, whereas Kelsh et al. classified the same study as being of lower quality. The Kelsh et al. meta-analysis included several new studies that appear to strengthen the finding of an increased risk of kidney cancer. Because of the limitations with both analyses, the committee concludes that neither should be used for hazard characterization in the risk assessment of trichloroethylene.

Recommendations: A new meta-analysis of the epidemiologic data on trichloroethylene and cancer should be performed to support a human health risk assessment. Techniques that would improve on past analyses include the following:

- Documenting the essential design features, exposure (either qualitative or quantitative), and results of the epidemiologic studies.
- Excluding studies based on objective criteria (e.g., studies in which it was unclear that the study population was exposed [e.g., studies of dry-cleaning workers]).
- Classifying studies in terms of objective characteristics, such as on the basis of the study's design characteristics or documentation of exposure.
- Combining case-control and cohort studies in the analysis, unless it introduces substantial heterogeneity.
- Testing of heterogeneity (e.g., fixed or random effects models).
- Performing a sensitivity analysis in which each study is excluded from the analysis to determine whether any study significantly influences the findings.

Toxicity and Cancer

Trichloroethylene is metabolized in the body by two major pathways: the oxidative pathway and the glutathione-conjugation pathway. The metabolites these pathways generate are thought to be responsible for the toxicity and carcinogenicity observed in different organ systems. Key scientific issues for characterizing these hazards include identifying the metabolites responsible for the effects, elucidating the mode of action, and understanding the relevance of animal data for humans.

Kidney Toxicity and Cancer

Trichloroethylene and some of its metabolites in the glutathione-conjugation pathway have been shown to be nephrotoxic and nephrocarcinogenic. There is concordance between animal and human studies. In bioassays, rats developed tubular toxicity before they developed tumors. Investigations of nephrotoxicity in human populations show that highly exposed workers exhibit evidence of damage to the proximal tubule. The magnitude of exposure needed to produce kidney damage is not clear.

Trichloroethylene nephrotoxicity is associated with a multistep metabolic pathway. It is generally accepted that the metabolite *S*-(1,2-dichlorovinyl)-L-cysteine is the penultimate nephrotoxicant. The metabolite can undergo bioactivation by conjugation to reactive species that

are genotoxic and cytotoxic and by sulfoxidation. Sulfoxides are more potent nephrotoxicants than their parent *S*-conjugates. Both *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide appear to play a role in renal tubular cell toxicity.

Evidence from experimental, mechanistic, and epidemiologic studies supports the conclusion that trichloroethylene is a potential kidney carcinogen. In animal studies, the nephrocarcinogenic effects of trichloroethylene were more pronounced in male rats than in female rats and were absent in male and female mice. Studies on trichloroethylene metabolism in rodents and in humans indicate a bioactivation role in the development of nephrocarcinogenicity. This has been linked with the formation of *S*-(1,2-dichlorovinyl)-L-cysteine; however, there are no studies of the carcinogenic potential of this metabolite.

Animal studies show that trichloroethylene acts as a complete carcinogen (at the stages of both tumor initiation and promotion and progression) in a dose-dependent manner, with nephrotoxicity as the promoter for cells initiated by a trichloroethylene metabolite. It is not possible to predict whether humans are more or less susceptible to the carcinogenic effects than other animals, because species differences in the extent of formation of *S*-(1,2-dichlorovinyl)-L-cysteine have not been fully characterized. Furthermore, the cytochrome P-450 enzyme isoforms that metabolize trichloroethylene have polymorphisms within national populations, resulting in considerable interindividual differences in enzyme expression. The committee ruled out the accumulation of $\alpha_{2\mu}$-globulin, peroxisome-proliferator activated receptor α (PPARα) agonism, and formic acid production as modes of action for the production of renal tumors in rodents.

Renal clear cell carcinoma, the carcinoma most often induced by trichloroethylene, was shown to link with the homozygous inactivation of the von Hippel-Lindau (*VHL*) tumor suppressor gene. The evidence indicates a strong association between trichloroethylene and *VHL* mutation, especially in protein expression, and kidney cancer in humans. Some studies have reported increased occurrence of mutations in renal cancer cells of patients exposed to high concentrations of trichloroethylene. The genotoxic effect of trichloroethylene metabolites likely results from bioactivation pathways in the kidney leading to renal *VHL* gene damage and renal cell carcinomas. However, there remains a lack of direct evidence that alterations in the *VHL* gene initiate renal tumors, but the alterations, especially in protein expression, might contribute to tumor progression. In the absence of information on the temporal relationship between *VHL* mutations and renal tumor initiation, it is prudent to assume that trichloroethylene-induced *VHL* mutations are initiating events. Direct evidence of alterations in the *VHL* gene in association with tumor progression remains to be determined.

Liver Toxicity and Cancer

Animal data on trichloroethylene indicate that relatively high doses are needed to induce liver toxicity and cancer, even in susceptible strains of mice. The three major oxidative metabolites of trichloroethylene—trichloroacetic acid, dichloroacetic acid, and chloral hydrate—can contribute to liver toxicity and cancer in rodents. Trichloroethylene produces hepatotoxicity in experimental animals and humans that depends on generation of reactive intermediates by the enzyme cytochrome P-450 in the liver. Studies with laboratory animals indicate that trichloroethylene and its metabolites also produce liver effects independent of hepatotoxicity, including elevation in plasma bile acid concentration and accumulation of liver glycogen. The relevance and significance of these effects to humans remain to be elucidated.

Trichloroethylene, chloral hydrate, and trichloroacetic acid induce liver cancer in mice when blood concentrations achieve millimolar concentrations. In contrast, dichloroacetic acid is active in rats as well and requires a much lower concentration to produce liver tumors. Trichloroethylene and its metabolites promote liver cancer. The mode of action for trichloroacetic acid in liver is principally as a liver peroxisome proliferator and agonist of PPARα rather than as a genotoxicant. A significant lack of concordance in the sensitivity of human and rodent hepatocytes to peroxisome proliferators and early events associated with liver tumor promotion has been noted, with humans being much less sensitive. In addition, there is no supporting epidemiologic evidence of enhanced occurrence of liver tumors in humans administered potent rodent peroxisome proliferators. The weak carcinogenic activity in the liver of chloral hydrate in male $B6C3F_1$ mice combined with lower rates of oxidation and higher rates of conjugation in humans compared with mice indicate that the mode of action for mice is not relevant to humans.

Species differences in susceptibility and phenotypic differences in tumors derived from trichloroethylene and its metabolites suggest that there are mechanistic differences in the way these chemicals cause tumors that cannot be fully explained by peroxisome proliferation. In rodents, the promotional activity of dichloroacetic acid includes a significant effect on cellular metabolism and cellular proliferation that encompasses a mitogenic mode of action. Assuming a mitogenic mode of action for dichloroacetic acid as a rodent liver carcinogen, genotypic species differences between mice and humans suggest that humans would be much less susceptible to liver carcinogenesis.

Reproductive and Developmental Toxicity

Evidence from animal and epidemiologic studies suggests that several reproductive and developmental toxicity end points may be associated with trichloroethylene exposure, including infertility in males and females, impaired fetal growth, and cardiac teratogenesis. Multiple rodent studies indicate that trichloroethylene affects spermatogenesis and the fertilizing capability of sperm in males and decreased fertilizability of oocytes in females. The effects appear to depend on metabolic activation of trichloroethylene by CYP2E1, but which oxidative metabolite is the proximate toxicant remains unknown. The relevance of these effects on rodent reproduction for predicting human outcomes also is not clear.

Multiple animal studies have found decreased fetal growth after maternal exposure to trichloroethylene. Impaired fetal growth was also a consistent finding in different community studies of mothers exposed to drinking water contaminated with trichloroethylene or tetrachloroethylene, a compound that has some of the same metabolites as trichloroethylene. However, a mechanistic basis for this effect remains to be elucidated.

Multiple studies in mammalian and avian models suggest that trichloroethylene or one or more of its metabolites (trichloroacetic acid and dichloroacetic acid) can cause cardiac teratogenesis. The avian studies are the most convincing. Rodent studies have had mixed results, suggesting either methodological or strain differences. The committee noted that the low-dose studies showing a positive correlation in trichloroethylene-induced cardiac teratogenesis showed unusually flat dose-response curves and came from a single laboratory. The results need to be replicated in another laboratory to clarify the dose-response relationship.

Epidemiologic investigations of communities exposed to trichloroethylene have also reported mixed results. A 2- to 3-fold increase in risk of congenital heart defects was found in multiple studies, and the most frequently found defects were the same in animal and human studies (defects of the interventricular septae and the valves). In addition, mechanistic support is provided by studies in animals demonstrating altered proliferation in the endocardial cushions at low dose or alterations in endothelial cell activation and decreased expression of two markers of epithelial mesenchymal cell transformation, a key process in valve and septum formation. Evidence that trichloroacetic acid and dichloroacetic acid are as potent as trichloroethylene suggests that CYP2E1 metabolic activation, as well as the fractional formation of trichloroacetic acid from chloral, is important in trichloroethylene cardiac teratogenesis.

Neurotoxicity

Past evidence showed that inhalation of trichloroethylene causes neurotoxic effects in laboratory animals and humans that are similar in nature (e.g., massiter reflex latency, motor incoordination, changes in heart rate) and occur at comparable concentrations of exposure (7-16 parts per million [ppm]). New information has not added substantially to the understanding of these effects. In particular, there continue to be a lack of data for understanding the effects of chronic exposure to trichloroethylene. It is not yet possible to ascertain the extent of trichloroethylene-induced impairment of complex neurological functions such as learning, memory, and attention. Whether there is preferential vulnerability to trichloroethylene across these domains, what exposure parameters might be associated with the effects, the extent of their reversibility, and the impact of the developmental period of exposure on such effects remain to be elucidated. It has been suggested that exposure to trichloroethylene during early development could enhance its effects on the nervous system, but the available data are insufficient to draw firm conclusions. Aging appears to enhance susceptibility of the nervous system after exposure to trichloroethylene. Some studies suggest a contribution of trichloroethylene to Parkinson's disease. Multiple mechanisms appear to contribute to the neurotoxic action of trichloroethylene, and further study is needed to elucidate them more precisely.

Respiratory Toxicity and Cancer

Trichloroethylene has been shown to induce lung tumors in rodents. It is well documented that the mode of action for this effect is localization of cytochrome P-450 metabolites of trichloroethylene in the Clara cells of the lungs and that pulmonary metabolism of trichloroethylene is species dependent. The proximate toxicant for the Clara cell, whether chloral, dichloracetyl chloride, or another metabolite, is still under study. The collective evidence indicates that rodents and humans are significantly different in their capacity to metabolize trichloroethylene in the lungs, with humans having less capacity. Results of most epidemiologic studies of occupational exposure to trichloroethylene do not show a strong association between trichloroethylene exposure and increased incidence of lung tumors. Thus, pulmonary cancer does not appear to be a critical end point in assessing human health risks to trichloroethylene.

Immunotoxicity

Among the immunotoxicity end points the committee evaluated, evidence for an effect of trichloroethylene was strongest for autoimmune disease. Studies in genetically susceptible rodents have shown that trichloroethylene exacerbates underlying autoimmune disease, and supporting information comes from multiple human studies of scleroderma and exposures to organic solvents. The metabolites and the mode of action involved have not been elucidated, but a role for chloral has been implicated in mouse models. Some individuals might be genetically susceptible to developing autoimmune disease; alterations in the CYP2E1 gene are suspected to play a role.

Susceptibility Issues

Several factors can contribute to an individual's susceptibility to the toxic effects of trichloroethylene, including disease states and differences in the expression of enzymes involved in metabolizing and disposing of trichloroethylene. For example, conditions such as alcoholism, obesity, and diabetes are known to induce the expression of CYP2E1, which is the rate-limiting enzyme of trichloroethylene metabolism. However, it is not known which human enzymatic isoforms are most efficient at disposing of trichloroethylene and its metabolites. This information is critical for understanding the relevance of various common functional genetic polymorphisms already known among enzyme families involved in trichloroethylene disposition, as well as those that might be identified in the future.

How to include human variability in risk assessments is an ongoing challenge. Traditionally, an array of uncertainty factors has been used to account for human variability, particularly for vulnerable populations. More precise estimates of the risk to susceptible subpopulations can be developed with the use of PBPK modeling developed for specific types of individuals. It appears that such modeling could be developed for the fetus and child. Not enough is known about other susceptibility factors to provide quantitative estimates of how these factors affect risk.

A formalized assessment of the quantitation of the dispositional differences associated with obesity, alcoholism, and coexposures also might allow similar models to be developed to better evaluate nondevelopmental differences in susceptiblity.

Mixtures

The available data indicate that toxic effects of trichloroethylene are likely to change in the presence of exposure to other chemicals, including its metabolites and similar metabolites of other toxicants. Clear understanding of whether and which of the toxic effects might be increased, decreased, or unchanged is lacking, but it appears that the major potential mechanisms of such interactions at the biophase include altered xenobiotic metabolizing enzymes, toxicokinetic factors (absorption, distribution, and elimination), toxic metabolite accumulation in target or nontarget tissues, and toxicodynamic factors, such as cell death, cell proliferation,

expression of survival factors, and epigenetic and genotoxic mechanisms. However, to what extent and how such factors influence toxicity outcomes cannot be predicted.

There is a large database on the interaction between ethanol and trichloroethylene, where both the metabolism and the pattern of toxicity by trichloroethylene are changed. The bulk of this information is from studies of laboratory animals, but some human data suggest that this interaction can occur with consumption of alcohol. The significance of these alterations in patterns of toxicity and cancer is currently unknown.

PBPK Modeling

Several PBPK models for trichloroethylene have been developed over the past few decades. Each successive model has attempted to incorporate new information on the scientific understanding of trichloroethylene metabolism and the mode of action of toxicity. Models that have received the most recent attention are the Fisher models, the Clewell model, and a "harmonized" model. Each model has strengths and limitations, as the designers have tried to balance model complexity and uncertainty.

The models EPA used in its draft risk assessment are the Fisher models, which were designed to focus on liver cancer in rats and humans, and the Clewell model, which is more complex and designed for covering liver toxicity and cancer, kidney toxicity and cancer, and lung cancer. A "harmonized" model has been developed as part of a joint effort between the U.S. Air Force and EPA.

This joint group also developed a description of the relevant uncertainties, variabilities, and errors for this modeling. Variability was described using an extension of the PBPK model to a population PBPK model. Random effects distributions in the population model formally described variability as a probability distribution. Uncertainty was characterized by taking a Bayesian perspective, where uncertainty about any unknown quantity is described as a probability distribution. The probability distribution conditional on the observed data is known as the posterior distribution. Markov chain Monte Carlo simulation was used to summarize the posterior distributions on parameters of interest. Errors in the reported numerical summaries were characterized with a standard diagnostic method. Overall, the committee found that the uncertainties, variability, and errors of the harmonized model were characterized appropriately.

None of the currently available PBPK models considers all possible routes of exposure to trichloroethylene (e.g., dermal) or dose metrics for all potential health end points (e.g., neurotoxicity, teratogenicity). The harmonized model is a reasonable extension of the Fisher and Clewell models and is the best model available, but the mode of action and appropriate dose metric for each health end point have not been established. Thus, it is appropriate to consider dose metrics generated from PBPK modeling along with other dose metrics that have been used in the past.

PBPK models do not resolve the uncertainty about the mode of action of trichloroethylene, but they can inform experimental designs for studying it. Better understanding of the mode of action of trichloroethylene will drive model elaboration.

Dose-Response Assessment

The key scientific issues related to the dose-response assessment for trichloroethylene include selecting the point of departure for low-dose extrapolation, methods for modeling from the point of departure to zero dose, and characterizing uncertainty and variability in estimates of cancer and non-cancer risk. To select the point of departure, estimates from continuous dose-response models are preferred to use of the lowest-observed-adverse-effect level (LOAEL) or the no-observed-adverse-effect level (NOAEL). It is important that criteria be established to determine whether certain toxicologic or epidemiologic data sets are suitable for modeling and that the modeling approach used be explained and justified. In the absence of modeled estimates, a LOAEL or NOAEL may be used.

For dose-response assessment for risks of cancer, EPA's guidelines call for selecting a point of departure from among modeled doses near the lower end of the observed range. Several response levels (e.g., 1%, 5%, and 10%) and dose metrics are available for performing such assessments, and it is important to consider all relevant ones and to provide a clear rationale for selecting the point of departure.

There are several approaches to extrapolating from the point of departure to zero, including linear and nonlinear methods. Much emphasis has been given to incorporating mode-of-action information on the carcinogenicity of trichloroethylene in such extrapolations. However, the committee notes that information on response variability among humans is required in addition to mode of action information to clarify the shape of the low dose-response curve in humans. The mode of action for trichloroethylene as a kidney carcinogen remains unclear and likely involves multiple pathways. None of the existing epidemiologic data is suitable as a primary means of quantifying cancer risks.

Recommendations:

- Several points of departure should be considered and compared when performing point-of-departure-based dose-response assessments for cancer and non-cancer end points.
- When modeled estimates are used as points of departure in cancer and non-cancer risk assessments, it is important that (1) criteria are established for determining which data sets are suitable for modeling, (2) the selected response level is justified or multiple response levels are modeled and compared, (3) dose-response models are clearly described, (4) different dose metrics are considered and compared to assess whether the choice of metric substantially affects the dose-response assessment, and (5) when animal data are modeled, the methods for estimating human-equivalent doses are specified.
- Toxicologic data should be used to fit the primary dose-response model(s), and the available epidemiologic data should be used only for validation. Because the available information is insufficient to determine the best dose-response model for trichloroethylene, the default linear extrapolation procedure suggested in EPA's cancer guidelines can be applied but should first be explicitly defined.

RESEARCH RECOMMENDATIONS

Following are recommendations for areas of medium- to long-term study to aid the agencies in setting a research agenda to advance understanding of the human health risks from

trichloroethylene. This information will allow for more precise estimates of risk but is not necessary at this time for performing a credible risk assessment.

Kidney Toxicity and Cancer:

- Studies of the formation of *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxides by human tissues (liver and kidney), the extent to which these reactions occur in vivo, the enzymes involved, and interindividual variability of these enzymes.
- Clarification of the toxicologic significance of trichloroethylene or *S*-(1,2-dichlorovinyl)-L-cysteine *S*-conjugate sulfoxidation products.
- Evaluation of the potential of missense specific mutations in the *VHL* gene contributing to tumor initiation and progression.
- Studies of nephrotoxicity in workers exposed occupationally to trichloroethylene using valid measures of exposure.
- Assessment of *VHL* gene mutations in relation to trichloroethylene exposure in populations from different geographic regions to validate the findings from existing assessments.

Liver Toxicity and Cancer:

- Elucidation of the significance of increased bile acids in relation to the hepatotoxic potential of trichloroethylene, as well as in relation to other systemic effects, and the significance of such changes in humans.
- Studies of trichloroethylene on glycogen accumulation to assess the significance of this effect and its relevance to humans.
- Determination of whether an autoimmune response might play a role in trichloroethylene-mediated liver disease.
- Determination of the metabolic pathway and yield for forming dichloroacetic acid from trichloroethylene either via trichloroacetic acid or other pathway(s).

Reproductive and Developmental Toxicity:

- Studies of trichloroethylene on sperm and oocytes and possible consequences on reproduction. Elucidation of the metabolites responsible for such effects.
- Determination of subpopulations at greatest risk as well as mechanisms for the putative gender and maternal age-based susceptibility for poor intrauterine growth.
- Evaluation of the relevant dose ranges and mode of action for trichloroethylene-induced developmental effects to determine the most appropriate species for human modeling. More information on metabolic activation in the avian model to evaluate interspecies differences, tissue-specific concentrations of trichloroethylene and its metabolites, and human data with better ascertainment of congenital heart disease and improved quantitative assessment of trichloroethylene exposures.

Neurotoxicity:

- Chronic exposure studies of the effects of trichloroethylene on the central nervous system to reduce reliance on short-term exposure data in risk assessments. Important research to pursue includes effects on functional end points, including cognitive deficits and motor and sensory function.
- Elucidation of the underlying mechanisms of trichloroethylene-induced neurotoxicity.

Immunotoxicity: Elucidation of the metabolites and modes of action by which trichloroethylene affects immunity and whether some individuals are genetically predisposed to developing autoimmune disease related to trichloroethylene exposure.

Susceptibility:

- Development of PBPK models for different physiologic stages of childhood development. Some research on children's exposure to trichloroethylene at different ages will be required to support model development (e.g., measurements of trichloroethylene metabolites in cord blood, breast milk, and meconium).
- Clarification of which human enzymatic isoforms are the most important in disposing of trichloroethylene and its metabolites.
- Better characterization of the impact of physiologic conditions and disease states on trichloroethylene toxicity.
- Evaluation of intersubject variation in pharmacodynamics across life stages and in various subpopulations is needed before pharmacodynamic factors can be quantitated in risk assessment. Before such pharmacodynamic data can be generated, the critical targets and modes of action must be clarified from animal or in vitro studies.

Mixtures:

- Toxicokinetic and toxicodynamic studies with mixtures to evaluate the effect of coexposures to other chemicals on toxic outcomes of trichloroethylene and its metabolites. Studies designed to evaluate modes of action in the presence of most commonly occurring toxicants are likely to yield more meaningful results than testing various combinations of compounds and doses.
- Testing the impact of lifestyle factors (e.g., alcohol consumption, chronic drug intake, caloric restriction), disease (e.g., diabetes), and special physiologic states (e.g., pregnancy, aging) on the toxicity of trichloroethylene.

PBPK Modeling:

- Future PBPK models for trichloroethylene risk assessment should include a description of dermal absorption.
- Studies to evaluate how well alternative dose metrics predict toxic response. PBPK models should be used to investigate alternative study designs.
- PBPK models should be developed for other toxicity end points, such as neurotoxicity and developmental outcomes. There may be little or no data available to confirm model predictions for certain tissue concentrations (e.g., brain) of trichloroethylene and metabolites in humans. However, inclusion of all relevant uncertainties can be formalized under Bayesian inference and implemented with Markov chain Monte Carlo approaches. Description of uncertainties in prior simulation might indicate that the approach is not practical without collecting additional data.
- Development of a combined PBPK model for trichloroethylene and ethanol.

1

Introduction

Trichloroethylene is a chlorinated solvent widely used as a degreasing agent in industrial and manufacturing settings. It is also used as a chemical intermediate in making other chemicals and is a component of products such as typewriter correction fluid, paint removers, adhesives, and spot removers (ATSDR 1997a). Trichloroethylene is released into the environment as the result of its use and disposal practices, primarily from vapor degreasing operations. It is also found in soils and surface waters as a result of direct discharges and in groundwater due to leaching from disposal operations.

Trichloroethylene is a common environmental contaminant at Superfund sites, Department of Defense facilities, and certain manufacturing operations (e.g., aircraft, spacecraft). It has been found at approximately 852 of the 1,416 sites proposed for inclusion on the U.S. Environmental Protection Agency (EPA) National Priorities List. On the basis of data reported to the EPA Toxic Release Inventory, it was estimated that approximately 42 million pounds of trichloroethylene were released into the environment in 1994 (Scott and Cogliano 2000).

In 2001, EPA issued a draft health risk assessment and proposed exposure standards for trichloroethylene. EPA's Scientific Advisory Board (SAB) reviewed the draft and it was issued for public comment. A number of scientific issues were raised during the course of these reviews by SAB, federal agencies, the scientific community, environmental organizations, citizen groups, and other interested parties. To help address these issues, EPA, the Department of Defense, the Department of Energy, and the National Aeronautics and Space Administration asked the National Research Council (NRC) to review and provide advice on the key scientific issues raised. In response to this request, NRC convened the Committee on Human Health Risks of Trichloroethylene, which prepared this report.

STATEMENT OF TASK

The committee was asked to identify and assess the key scientific issues relevant to analyzing the human health risks of trichloroethylene. In performing its task, the committee was asked to consider pertinent toxicologic, epidemiologic, population susceptibility, and other available information, including relevant published scientific literature, EPA's 2001 draft health

risk assessment of trichloroethylene, scientific and technical comments received by EPA from public and private sources, and additional relevant information to be provided by the sponsoring agencies. The committee was tasked with holding one or more information-gathering sessions open to the public to gain additional insights into the issues from federal agencies, concerned parties, and other scientists.

The committee was asked to highlight issues critical to the development of an objective, realistic, and scientifically balanced trichloroethylene health risk assessment. The focus was to be on hazard characterization and mode of action for trichloroethylene toxicity, possible approaches to synthesize epidemiologic data in informing the hazard characterization of trichloroethylene, differential susceptibility in different subpopulations or life stages, the evidence for effects from trichloroethylene exposures alone compared with that for effects from mixtures of chemicals that include trichloroethylene, physiologically based pharmacokinetic modeling, dose-response assessment, and quantitative assessment of cancer and non-cancer risks. The availability of appropriate data and methods to implement the committee's advice as well as the distinction between data analysis and data generation were to receive special attention. The committee was asked to distinguish between issues that can be addressed through short-term analyses and issues that are more appropriately addressed through medium- or long-term research projects.

The committee was not asked to develop its own risk assessment or to address any risk management issues.

COMMITTEE'S APPROACH

The committee held five meetings between March and November 2005. The first three meetings involved data-gathering sessions, where the committee heard from sponsors, invited speakers, representatives of citizen groups, and members of the public. The committee reviewed a large body of written material on trichloroethylene, including research articles, literature reviews, position papers, and unpublished data submitted by various sources, including the public. It focused its review on new data generated since EPA's 2001 draft risk assessment, pertinent older information, and their implications for conducting a scientifically balanced risk assessment.

The committee is aware that some readers expect this report to evaluate EPA's 2001 draft health risk assessment or to provide a comprehensive evaluation of the literature on trichloroethylene. However, the scope of this project did not encompass either of these tasks. The statement of task directed the committee to evaluate specific issues related to performing a risk assessment on trichloroethylene. EPA's draft assessment was to be factored into the committee's evaluation, but it was not to be the main focus of the review. Thus, although the report comments on certain aspects of the draft assessment, the scientific validity of the proposed standards for trichloroethylene is not assessed.

As directed in the statement of task, the committee's review of the different cancer and non-cancer end points focused primarily on mode-of-action information and how it contributes to the hazard characterization of trichloroethylene. With regard to cancer, the committee's evaluation focused on the assessment of kidney cancer. Guidance is provided on how to evaluate the epidemiologic evidence and to consider this information in conjunction with animal evidence and mechanistic data. The assessment provided for kidney cancer is intended to be a model for

how other types of cancer should be evaluated. For the other cancer and non-cancer end points, a qualitative review of some epidemiologic evidence is provided to give a sense of whether the animal evidence is supported by observations in human populations. However, these data sets were not rigorously reviewed.

OVERVIEW OF PHARMACOKINETICS

Understanding the absorption, distribution, metabolism, and elimination of trichloroethylene is critical to the qualitative and quantitative assessment of human health risks from environmental exposures. Qualitatively, pharmacokinetics is helpful in identifying the chemical species that might be causally associated with observed toxic responses. This is particularly important for trichloroethylene because many of its toxic effects are thought to be due to metabolites rather than to trichloroethylene. The delineation of interspecies and intraspecies pharmacokinetic differences can provide insights into how laboratory animal and epidemiologic data might reveal overall human health risks and the basis for individual differences in susceptibility. Furthermore, physiologically based pharmacokinetic models can quantify the relationship between external measures of exposure and internal measures of a toxicologically relevant dose. Selecting the appropriate dose metric for use in risk assessment depends on the understanding of the target tissue, active chemical, and mode of action for a particular toxic effect as well as the reliability of physiologically based pharmacokinetic models.

Trichloroethylene is rapidly and extensively absorbed by all routes of environmental exposures, including ingestion, inhalation, and dermal contact. Once absorbed, trichloroethylene distributes throughout the body via the circulatory system. Most trichloroethylene taken into the body is metabolized; direct exhalation of the parent compound is the other major route of elimination (Lash et al. 2000a).

Figure 1-1 presents a postulated scheme for the pathways of trichloroethylene metabolism, adapted from the work of Clewell et al. (2000), Lash et al. (2000a), and recent studies described below. Trichloroethylene metabolism occurs through two main, irreversible pathways—oxidation via the microsomal mixed-function oxidase system (cytochrome P-450s [CYPs]) primarily to chloral [C_2HCl_30]or chloral hydrate [$CCl_3CH(OH)_2$]and trichloroethylene oxide), and conjugation with glutathione by glutathione *S*-transferases to *S*-1,2-dichlorovinyl-L-glutathione. For trichloroethylene oxidation, CYP2E1 is thought to be most important in vivo. Subsequent important metabolic branch points include the production of trichloroethanol, regeneration of chloral and chloral hydrate from trichloroethanol, and further metabolism of *S*-1,2-dichlorovinyl-L-cysteine.

A number of important issues relate to understanding trichloroethylene pharmacokinetics: (1) enterophepatic recirculation of trichloroethylene and trichloroethanol; (2) diffusion-limited tissue distribution in fat and liver; (3) plasma binding of trichloroacetic acid and dichloroacetic acid; (4) dichloroacetic acid formation, pharmacokinetics, and the role of trichloroethylene oxide in its formation; and (5) pathways of gluthathione conjugation and subsequent metabolism. These and other pharmacokinetic considerations are presented in Appendix C.

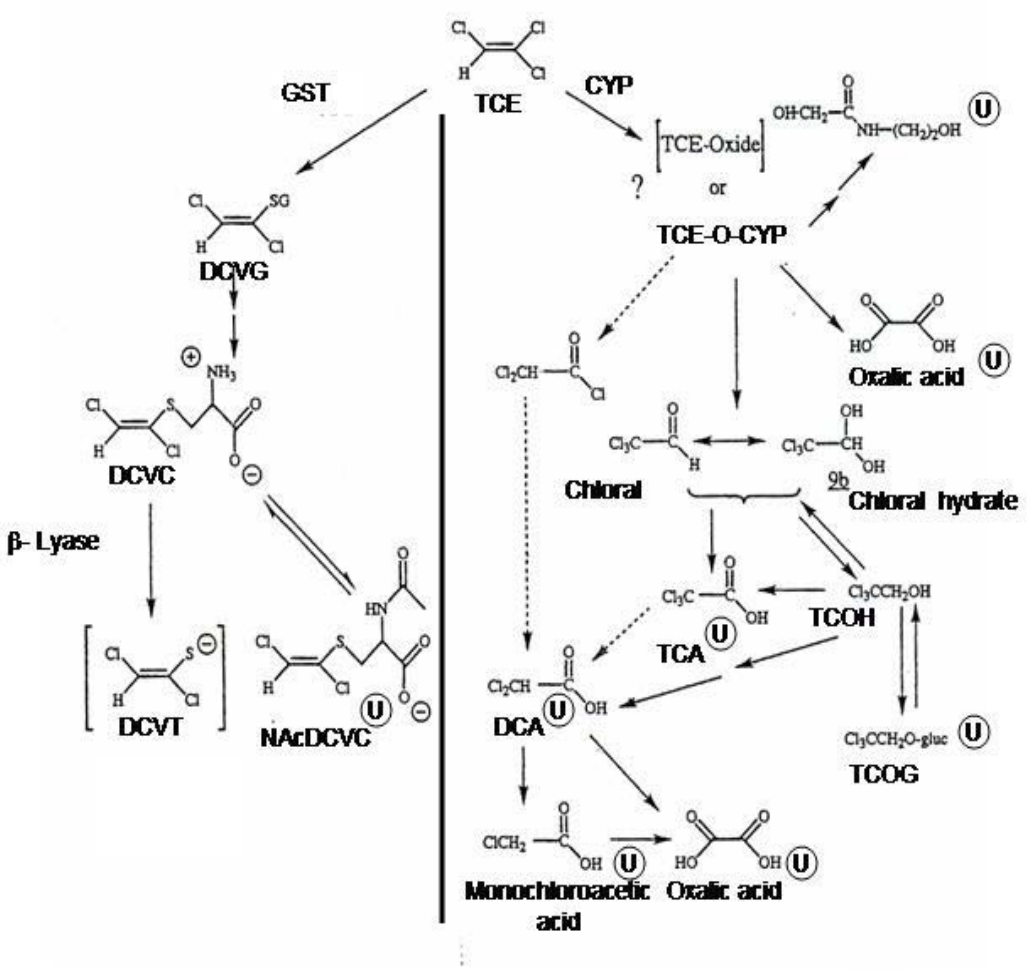

FIGURE 1-1 Metabolism of trichloroethylene. Metabolites marked with Ⓤ are known urinary metabolites. Arrows with broken lines indicate other possible steps in forming dichloroacetic acid. Abbreviations: CYP, cytochrome P-450; DCA, dichloroacetic acid; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVT, *S*-(1,2-dichlorovinyl)thiol; GST, glutathione *S*-transferase; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine; TCA, trichloroacetic acid; TCE, trichloroethylene; TCE-O-CYP, trichloroethylene-oxide-cytochrome P-450 complex; TCOH, trichloroethanol; TCOG, trichloroethanol glucuronide.
Source: Adapted from Lash et al. 2000a.

EXPOSURE CONSIDERATIONS

Trichloroethylene

People can be exposed to trichloroethylene from contaminated air (outdoor and indoor), water, and soil. Data from 2004 on ambient air concentrations of trichloroethylene indicate an average of 0.37 μg/m^3 (range, 0-6.32 μg/m^3), a concentration that has remained fairly consistent since 1996 (EPA, unpublished material, June 2005). Mean concentrations at various land-use sites include 1.84 μg/m^3 in commercial areas, 1.54 μg/m^3 in industrial areas, 1.08 μg/m^3 in

agricultural areas, and 0.89 μg/m^3 in residential areas. Trichloroethylene concentrations measured in ambient air at various U.S. locations are provided in Table 1-1.

Indoor air can become contaminated by certain consumer products (e.g., adhesives, tapes) and by volatilization from contaminated water supplies. Vapor intrusion through walls and floors can also be a source of indoor exposure when buildings are near contaminated groundwater. Information on measured concentrations of trichloroethylene in indoor air is presented in Table 1-2. Some of the studies used outdoor concentrations and personal samples for comparison.

Trichloroethylene is the most frequently reported organic contaminant in groundwater. The Agency for Toxic Substances and Disease Registry (1997) estimates that between 9% and 34% of drinking water supply sources tested in the United States contain some trichloroethylene. Example concentrations of trichloroethylene found in effluents, surface water, rainwater, groundwater, and drinking water are presented in Table 1-3. Groundwater concentrations of trichloroethylene have been extensively sampled in California. A statewide survey conducted in 1984-1985 found trichloroethylene in 187 of 2,947 wells at concentrations up to 440 μg/L (DHS 1986). The most contaminated wells were typically found in more urbanized areas.

TABLE 1-1 Concentrations of Trichloroethylene in Ambient Air

		Concentration, μg/m^3		
Area	Year	Mean	Range	Reference
Rural				
Whiteface Mountain, NY	1974	0.5	<0.3-1.9	IARC 1995a
Badger Pass, CA	1977	0.06	0.005-0.09	IARC 1995a
Reese River, NV	1977	0.06	0.005-0.09	IARC 1995a
Jetmar, KS	1978	0.07	0.04-0.11	IARC 1995a
Urban and Suburban				
New Jersey	1973-1979	9.1	ND-97	IARC 1995a
New York City, NY	1974	3.8	0.6-5.9	IARC 1995a
Los Angeles, CA	1976	1.7	0.14-9.5	IARC 1995a
Lake Charles, LA	1976-1978	8.6	0.4-11.3	IARC 1995a
Phoenix, AZ	1979	2.6	0.06-16.7	IARC 1995a
Denver, CO	1980	1.07	0.15-2.2	IARC 1995a
St. Louis, MO	1980	0.6	0.1-1.3	IARC 1995a
Portland, OR	1984	1.5	0.6-3.9	IARC 1995a
Philadelphia, PA	1983-1984	1.9	1.6-2.1	IARC 1995a
Southeast Chicago, IL	1986-1990	1.0	—	Sweet and Vermette 1992
East St. Louis, IL	1986-1990	2.1	—	Sweet and Vermette 1992
District of Columbia	1990-1991	1.94	1-16.65	Hendler and Crow 1992
Urban Chicago, IL	Pre-1993	0.82-1.16	—	Scheff and Wadden 1993
Suburban Chicago, IL	Pre- 1993	0.52	—	Scheff and Wadden 1993
300 cities in 42 states	Pre-1986	2.65	—	Shah and Singh 1988
Several Canadian cities	1990	0.28	—	Bunce and Schneider 1994
Several US cities	1990	6.0		Bunce and Schneider 1994
Phoenix, AZ	1994-1996	0.29	0-1.53	Zielinska et al. 1998
Tucson, AZ	1994-1996	0.23	0-1.47	Zielinska et al. 1998

Source: EPA, unpublished material, June 2005.

TABLE 1-2 Example Concentrations of Trichloroethylene in Indoor Air

	Mean Trichloroethylene Concentration, μg/m^3			
Setting	Indoor	Outdoor	Personal	Reference
Baltimore Harbor Tunnel toll booths[a]	3.11	0.08		Sapkota et al. 2005
Residential, Ottawa, Canada	0.06	0.08		Zhu et al. 2005
Residential, Minnesota (Children's Pesticide Exposure Study)	0.6	0.6	0.8	Adgate et al. 2004
Residential, Minneapolis/St. Paul, Minnesota	0.5	0.2	1.0	Sexton et al. 2004
U.S., Canada, Europe	<1-165			Hers et al. 2001
Residences and workplaces in the United States	7.2			Shah and Singh 1988
Bathrooms in two homes using trichloroethylene-contaminated well water	500-40,000			Andelman et al. 1986

[a]Dry-cleaning residues on uniforms is thought to be the source of exposure

TABLE 1-3 Concentrations of Trichloroethylene in Water

	Location (No.		Trichloroethylene Concentrations, μg/L			
Water Type	of Samples)	Year	Mean	Median	Range	Reference
Industrial effluent	U.S.	1983		0.5		IARC 1995a
Surface water	U.S.	1983		0.1		IARC 1995a
Rainwater	Portland, OR	1984	0.006		0.002-0.02	Ligocki et al. 1985
Groundwater	MN	1983			0.2-144	Sabel and Clark 1984
	NJ	1976			≤1,530	Burmaster 1982
	NY	1980			≤3,800	Burmaster 1982
	PA	1980			≤27,300	Burmaster 1982
	MA	1976			≤900	Burmaster 1982
	AZ	—			8.9-29	IARC 1995a
Drinking water	U.S.	1976			0.2-49	IARC 1995a
	U.S.	1977			0-53	IARC 1995a
	U.S.	1978			0.5-210	IARC 1995a
	MA	1984			Max. 267	IARC 1995a
	NJ (1,130)	1984	23.4		Max. 67	Cohn et al. 1994
	CA (486)	1985			8-12	EPA 1987
	CA (486)	1984	66			EPA 1987
	NC (48)	1984	5			EPA 1987
	ND (48)	1984	5			EPA 1987

Source: EPA 2001a.

Metabolites

Trichloroethylene toxicity comes primarily from its metabolites, but people may be exposed to the metabolites from sources other than trichloroethylene. For example, chlorination

of drinking water produces the by-products chloral, chloral hydrate, monochloroacetic acid, dichloroacetic acid, and trichloroacetic acid. Chloral is used in the production of polyurethanes and as a chemical intermediate for the herbicide trichloroacetic acid. Chloral hydrate is a pharmaceutical used as a hypnotic and sedative. The metabolite monochloroacetic acid is used in pharmaceuticals, as an herbicide, and as a chemical intermediate in the production of indigoid dyes. Trichloroacetic acid is also used as a chemical intermediate and in the production of herbicides (EPA 2001a).

Other chemical compounds have some of the same metabolites as trichloroethylene, including tetrachloroethylene, 1,1,1-trichloroethane, 1,2-dichloroethylene (*cis*-, *trans*-, and mixed isomers), 1,1,1,2-tetrachloroethane, and 1,1-dichloroethane. Tetrachloroethylene is used in textile dry cleaning, as part of the processing and finishing in cleaning and degreasing metals, and as a chemical intermediate in the synthesis of some fluorocarbons. 1,1,1-Trichloroethane is used as a solvent and in pesticides, textile processing, cutting oil formulations, and printing inks. 1,2-Dichloroethylene, 1,1,1,2-tetrachloroethane, and 1,1-dichloroethane are used primarily as solvents in cleaning, degreasing, and extracting processes (EPA 2001a). EPA's preliminary set of dose estimates for these compounds and the metabolites they share with trichloroethylene are presented in Table 1-4.

ORGANIZATION OF THE REPORT

Guidance for hazard characterization of trichloroethylene is presented in Chapters 2 through 10. Chapter 2 provides guidance for evaluating large sets of epidemiologic data. In Chapter 3, the committee applies this guidance as an example in its evaluation of the epidemiologic data on trichloroethylene and kidney cancer, and this example should help guide evaluations of other cancer risks. Chapter 3 also assesses new information on the kidney toxicity of trichloroethylene and its metabolites and potential modes of action. Chapters 4, 5, 6, 7, and 8 evaluate the key issues regarding liver toxicity and cancer, reproductive and developmental toxicity, neurotoxicity, respiratory tract toxicity and cancer, and immunotoxicity, respectively. While these chapters are divided by target organ site, it is important to note that concordance of target organ effects in animals and humans was not a requirement in evaluating the implications of animal data for human risk. Site concordance of tumors is usually judged as providing strong evidence of an association, and the committee did consider this relationship in evaluating the data. However, the committee's review focused on mode of action information to understand how trichloroethylene might affect certain processes differently in different species. Chapter 9 discusses susceptibility to trichloroethylene and its metabolites, and Chapter 10 describes important factors in considering trichloroethylene in mixtures. Physiologically based pharmacokinetic models are evaluated in Chapter 11, and guidance is provided on future directions for model development. Finally, Chapter 12 considers issues related to dose-response assessment and quantitative assessment of risk.

TABLE 1-4 Preliminary Intake Estimates of Trichloroethylene and Related Chemicals

Chemical	Population	Media	Range of Estimated Adult Exposures, µg/day	Range of Adult Doses, mg/kg/day	Data Sources
Trichloroethylene	General	Air	11–33	1.57E-04-4.71E-04	ATSDR 1997a
	General	Water	2–20	2.86E-05-2.86E-04	ATSDR 1997a
	Occupational	Air	2,232-9,489	3.19E-02-1.36E-01	ATSDR 1997a
Tetrachloroethylene	General	Air	80–200	1.14E-03-2.86E-03	ATSDR 1997b
	General	Water	0.1-0.2	1.43E-06-2.86E-06	ATSDR 1997b
	Occupational	Air	5,897-219,985	8.43E-02-3.14	ATSDR 1997b
1,1,1-Trichloroethane	General	Air	10.8–108	1.54E-04-1.54E-03	ATSDR 1995
	General	Water	0.38-4.2	5.5E-06-6.00E-05	ATSDR 1995
1,2-Dichloroethylene	General	Air	1-6	1.43E-05-8.57E-05	ATSDR 1996
	General	Water	2.2	3.14E-05	ATSDR 1996
Cis-1,2-Dichloroethylene	General	Air	5.4	7.71E-05	HSDB 1996[a]
	General	Water	0.5-5.4	7.14E-06-7.71E-05	HSDB 1996[a]
1,1,1,2-Tetrachloroethane	General	Air	142	2.03E-03	HSDB 2002[b]
1,1-Dichloroethane	General	Air	4	5.71E-05	HSDB 2002[b]
	General	Water	2.47-469.38	3.53E-05-6.71E-03	ATSDR 1990
Chloral	General	Water	0.02-36.4	2.86E-07-5.20E-04	HSDB 1996
Monochloroacetic acid	General	Water	2-2.4	2-86E-05-3.43E-05	EPA 1994a
Dichloroacetic acid	General	Water	10-266	1.43E-04-3.80E-03	IARC 1995a
Trichloroacetic acid	General	Water	8.56-322	1.22E-03-4.60E-03	IARC 1995a

[a]Data from Hazardous Substances Data Bank 1996.

[b]Data from Hazardous Substances Data Bank 2002 (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB).

Source: EPA 2001a.

2

Methodological Considerations in Evaluating the Epidemiologic Literature on Cancer and Exposure to Trichloroethylene

There are numerous epidemiologic investigations available on cancer outcomes and exposure to trichloroethylene. How to consider the findings of multiple studies that differ in design, quality, and outcome has been identified as one of the critical aspects of conducting a hazard characterization of trichloroethylene. In this chapter, the committee provides generic guidance on evaluating epidemiologic studies on trichloroethylene, including guidance on identifying relevant epidemiologic studies, evaluating their strengths and weakness, and qualitative methods for evaluating the data (e.g., the Hill [1965]s guidelines on assessing causality). Quantitative methods for combining and summarizing epidemiologic data (i.e., meta-analytical approaches) are discussed, and a review is provided of two available analyses that used such quantitative approaches to evaluate the data. The chapter provides targeted recommendations for how those quantitative assessments can be improved upon in a new meta-analysis. An example of how this chapter's guidance should be applied is provided in the committee's assessment of the epidemiologic literature on kidney cancer presented in Chapter 3, and should also be applied to other outcomes (see Chapters 3-8). An important area of future review will be lymphoid cancers, particularly non-Hodgkin's lymphoma and childhood leukemia, which were topics the committee was unable address during the course of its study.

HEALTH OUTCOMES

Epidemiologic studies of etiology are used to answer questions about whether antecedent exposures in populations increase the risk of developing specific health outcomes. A variety of health outcomes associated with trichloroethylene is discussed in Chapters 3 to 8. At least three levels of health outcomes should be considered in assessing the human health risks associated with exposure to trichloroethylene: biomarkers of effects and susceptibility, morbidity, and mortality.

Few known susceptibility biomarkers specific to trichloroethylene have been assessed in humans. In the case of liver toxicity (see Chapter 4), incipient effects on the liver could be measured by changes in liver enzymes in the serum, although significant toxicity would have to be present for these measurements to be useful. Assessment of immune function may have a place in assessing adverse effects of trichloroethylene (see Chapter 8), but this outcome is nonspecific (Iavicoli et al. 2005). Human studies on proteinuria and other early markers of kidney toxicity are important (see Chapter 3). However, none of these potential biomarkers is specific to trichloroethylene.

High occupational or accidental exposure to trichloroethylene can produce toxicity, in particular, liver and central nervous system effects. The public-health review process focuses on more subtle effects resulting from exposures to lower concentrations. These morbidity outcomes can be in the form of cancer and non-cancer outcomes. Many non-fatal, non-cancer health end points are poorly measured and the few studies are difficult to interpret, mostly because current health monitoring systems are not set up to easily link health outcome data to exposure. On the other hand, cancer incidence is enumerated much more accurately by tumor registries, which usually have high diagnostic accuracy (histologic assessment of tumor location and tumor type). Alternatively, histologically confirmed cases of cancer (except for nonmelanotic skin cancer) can be identified through records in hospital pathology departments, which may be useful in two ways. First, they provide the cases for case-control studies, the method of choice to assess rare tumors, such as childhood cancers. Second, they match cohorts to tumor registries where the cohort members reside.

Mortality is readily identified from death certificates, which are collected routinely on a jurisdictional basis (e.g., state or province) and collated nationally. This outcome has the advantage of having complete national coverage but diagnostic accuracy is reduced because the attending physicians who fill out the certificates usually do not have the benefit of histologic diagnosis or autopsy findings. Most cohort studies rely on mortality data for risk assessment. It must be recognized that diagnostic accuracy from death certificates varies by the specific diagnosis (Brenner and Gefeller 1993).

Disease classification systems are also periodically revised, adding to diagnostic inconsistency (Irons 1992). The issue of changes in diagnostic coding systems is illustrated for the classification of lymphatic and hematopoietic cancers (the non-Hodgkin's lymphomas). As noted by the Institute of Medicine (IOM 2003), revisions 7 and earlier of the International Classification of Diseases did not have specific rubrics for some diseases, such as acute leukemia, but did have codes for lymphosarcoma and reticulosarcoma (ICD-200), Hodgkin's disease (ICD-201), and lymphatic leukemia (ICD-204). Because of the lack of numbers for specific types of tumors, in older cohort studies

> all lymphatic and hematopoietic neoplasms were grouped together instead of handled as individual types of cancer (such as Hodgkin's disease) or specific cell types (such as acute lymphocytic leukemia). The amalgamation of these relatively rare cancers would increase the apparent sample size but could result in diluted estimates of effect if the different sites of cancer were not associated in similar ways with the exposures of interest. In addition, before the use of immunophenotyping to distinguish ambiguous diseases, diagnoses of these cancers may have been misclassified; for example, non-Hodgkin's lymphoma [NHL] may have been misclassified as Hodgkin's disease [HD] [Irons 1992]. Misclassification of specific types of cancer, if unrelated to exposure,

> would have attenuated estimates of relative risk and reduced statistical power to detect associations. When the outcome was mortality, rather than incidence, misclassification would be greater because of the errors in the coding of underlying causes of death on death certificates (IOM 2003, p. 282).

Thus, older studies that combined all lymphatic and hematopoietic neoplasms must be interpreted with care.

Age and gender, two important factors influencing outcome, must be considered when assessing the risks associated with exposure to trichloroethylene. Cancer incidence varies widely by age; for example, children have different leukemia subtypes than adults. Age likely influences susceptibility to a number of environmental toxic materials both directly and indirectly through behavioral patterns, such as indoor-outdoor times, respiratory ventilation rates, and eating habits.

Men and women have obvious differences in disease outcomes epitomized by diseases affecting sex organs. Again there are innate differences as well as differences that might be attributable to behavioral and environmental factors, such as exercise and occupation. For evaluating childhood disease risk, one must consider transmission of risk from the mother or the father. Obvious gender differences are in play again, such as in utero exposure and exposure to toxins in mother's breast milk. Risk from germinal transmission could apply to either parent but again differences exist between ova and sperm formation, allowing potential differences in transmissible toxic risks. Such issues related to trichloroethylene are presented in Chapters 5 and 9.

DESIGNS OF EPIDEMIOLOGIC STUDIES

The main study designs used in epidemiology to assess etiology are the cohort study and the case-control study; other designs used in epidemiology are case studies, ecologic studies, and cross-sectional studies. The cohort and case-control study designs can provide sufficiently high-quality data to determine whether there are associations between sites of cancer and previous exposure to trichloroethylene. Assessing causality from such associations can then be considered if there has been a suitable exposure assessment and if bias can be eliminated as a reason for observing these associations.

Case studies (or case series) are not useful for estimating exposure-response relationships, because they do not make use of a reference population and therefore do not provide estimates of risk, incidence, or mortality rates. Case studies may be useful for developing hypotheses and may have some relevance for identifying hazards, particularly when a disease is extremely rare (e.g., angiosarcoma and vinyl chloride) and a few cases in a population with a common exposure may suggest an increased risk.

Ecologic studies are used to estimate correlations between rates of cancer in geographically circumscribed populations and exposure measured at the geographic level. It is important to distinguish between "pure" ecologic studies and other types of analytic studies, which have data on an individual level but make use of an exposure variable that is assigned uniformly to all subjects in specific areas. In the latter types of studies, which are not to be classified as ecologic studies; it is assumed that it is valid to assign one level of exposure to all subjects in a geographic area, although there may be some inherent misclassification because not

all are exposed uniformly. If the measurement error is independent of geographic area (e.g., county), then risk estimates will usually be attenuated.

For the pure ecologic studies of end points in which there are no individual data and there are other important risk factors, the main methodological issue is bias from uncontrolled confounding (referred to as the "ecologic fallacy" or cross-level bias). This bias may occur because an association observed between variables measured on an aggregate level does not necessarily represent an association at the individual level (see Morgenstern 1998). A quintessential example is found in the literature on radon and lung cancer, where rates of lung cancer in U.S. counties showed a negative association with average concentrations of radon measured in the counties (Cohen and Colditz 1994), whereas the individual case-control and cohort studies show positive exposure-response patterns (NRC 1988). The cross-level bias in this example is likely due to the nonlinear exposure response measured on an individual level and to confounding by smoking (Greenland and Robins 1994). For diseases with only one major risk factor, ecologic studies may provide accurate estimates of risk at the individual level; for example, the original study by Snow (1856) on cholera in London was ecologic and was not subject to the ecologic fallacy because cholera has only one cause.

Cross-sectional studies provide a snapshot of the prevalence, but not incidence, of health conditions in a specific population at one point, or over a short period, in time. The prevalence of cancer in subjects who may or may not have been exposed to trichloroethylene can be compared as prevalence proportions. Incidence rate ratios (or differences), the main etiologic parameters of interest, cannot be estimated if the prevalence of disease is related to the duration of disease. Cross-sectional studies are rarely useful for studying cancer because of this issue and because of possible selection biases in the underlying cohort that provides the sources of the population (e.g., selection of study participants to assess the prevalence of kidney cancer that may be related to duration of cancer and also to exposure).

The cohort study is the principal methodological paradigm describing all analytical epidemiologic study designs in that the other designs differ from the cohort study only in the way subjects are sampled from an explicitly defined or an implicitly defined cohort. Explicitly defined cohorts include, for example, occupational populations for which a roster is established and subjects are followed over time. In this type of study, incidence (mortality) rates are estimable directly from following the population through time, thereby assessing vital status as well as the health outcomes of interest; these rates can be compared by the estimated exposure, adjusting for potential confounding factors. Exposure can be defined at the beginning of follow-up (or earlier, say at the beginning of employment) or reevaluated through time. In principle, other risk factors can also be assessed so that confounding bias can be eliminated through statistical adjustments. The nested case-control design is used usually to reduce the costs of obtaining information not available on the cohort roster (e.g., smoking information) and incidence density sampling is used to produce odds ratios that are unbiased estimators of the rate ratio (although they usually have larger standard errors than a full cohort analysis). The nested case-control study has major advantages for exposure assessment because only a sample of subjects needs to be assessed. In some cases, the exposure histories of the cohort have been determined by an exposure assessment, and then the nested case-control study is usually involved to assess secondary data that may confound the exposure effect. The nested case-control study is not to be confused with population- or hospital-based case-control studies that are used to select subjects from the general population or a subset of the general population; for example, the nested case-control study by Greenland et al. (1994), discussed in Chapter 3, should

be classified as a cohort study because the odds ratio, estimated from incidence density sampling, is an unbiased estimate of the hazard ratio.

Implicit cohorts are the basis for case-control studies in the general population, where cases and controls are selected from an underlying population. Often, incidence density sampling is used, as in nested case-control studies, but again the underlying cohort is not enumerated. Statistically, these studies are tremendously powerful, because the number of cases in principle can be maximized by increasing the intake period or using other geographic regions. A main methodological challenge with the case-control study is the definition of the population-based or nonnested control population (Wacholder et al. 1992a,b).

A general weakness of population-based case-control studies is the quality of the exposure information. A wide range of exposures in the general population can be difficult to characterize, and exposures of interest may have low prevalences, leading to low statistical power to detect effects. Frequently, the source of exposure information in these studies comes from interviews or information from secondary sources such as occupation on death certificates. A strength of the study design is that covariates can usually be measured but, like the exposure of interest, there may be misclassification if these occurred in the distant past. To make information from the case group comparable to the control group, special methods for obtaining information are used, including using a control population that also has some pathology (e.g., cancer controls for a study on breast cancer); using independent evaluators to assess exposure based on job descriptions (Siemiatycki et al. 1981, 1987; Stewart and Stewart 1994; Stewart et al. 1998); and defining in advance rules to indicate exposure based on job and industry classifications (referred to as job exposure matrices) (Hoar et al. 1980; Hsieh et al. 1983; Sieber et al. 1991; Bouyer and Hemon 1993; Dosemeci et al. 1994).

Thus, the two main designs most useful for risk assessment are cohort studies and case-control studies. However, judging the validity of a study solely in terms of type of design may be misleading. It has often been said that the cohort study is superior to the case-control study because data are collected so that the temporal chain in causality is clear and unambiguous. This may be true for prospective cohort studies in which exposure and other important variables are assessed prospectively, but a well-designed case-control study may be as informative as a well-designed retrospective cohort study. For example, in retrospective cohort studies, in which past exposure is inferred from various data sources, exposure misclassification may be as great as in population-based or hospital-based case-control studies. In addition, bias from the misclassification of disease may be introduced in cohort studies in which mortality is used as the end point, particularly for non-cancer outcomes, and such studies may be inferior to a well-conducted case-control study in which disease status is confirmed through rigorous means (e.g., in studies of cancer with histologic confirmation). Another issue with cohort studies, unless they are very large, is that the statistical power to detect small or moderate associations is diminished for rare outcomes.

The validity of any study may be difficult or impossible to verify, although some fundamental principles may help guide the way individual studies are evaluated. The U.S. Environmental Protection Agency (EPA) has provided a list of features that need to be evaluated and the committee largely agrees with this list:

> (1) clear articulation of study objectives or hypothesis; (2) proper selection and characterization of comparison groups (exposed and unexposed groups or case and control groups); (3) adequate characterization of exposure; (4) sufficient length of follow-

> up for disease occurrence; (5) valid ascertainment of the causes of cancer morbidity and mortality; (6) proper consideration of bias and confounding factors; (7) adequate sample size to detect an effect; (8) clear, well-documented, and appropriate methodology for data collection and analysis; (9) adequate response rate and methodology for handling missing data; and (10) complete and clear documentation of results. No single criterion determines the overall adequacy of a study (EPA 2005a, p. 2-4).

To this list can be added the notions of definition of the target population (all inferences are made to this population), selection of subjects (e.g., response rates, attrition rates), and statistical variation in the estimates of association. In the end, the judicious use of relevant epidemiologic studies will determine, using weight-of-the-evidence arguments (inductive reasoning), whether there is an association and, in conjunction with other data, whether the association may be causal.

EXPOSURE ASSESSMENT

A critical component of any epidemiologic study is the method used to assess exposure as well as its accuracy (validity and reliability) (see Smith 2002; Nieuwenhuijsen 2003). Figure 2-1 shows the basic levels of exposure assignments that may result from an exposure assessment and how they are related. Assignment of exposure is implicitly quantitative. The true underlying exposure intensity distribution on the right is highly skewed, with only a small fraction having high exposures. The fundamental exposure classification is to identify which members of a population are "exposed," and the term "exposure" may have several definitions (see below). The qualitative judgment about an agent being present in a subject's environment is based on information about the setting, which can be descriptive about the location, activities, agents that are or might be present, and data on local contamination of air, water, and food.

Definitions of Exposed

The most commonly used epidemiologic definitions of exposure are (1) a subject is potentially exposed because he or she spends some time in a setting where the agent is known to be present; (2) there is reasonable probability of exposure to the agent by inhalation, skin contact, or ingestion because of a subject's activities (e.g., job contact, water ingestion); (3) potentially exposed subjects have at least a minimum amount of the agent present in personal samples (e.g., skin contamination) or biological samples (e.g., blood, urine). Clearly these samples do not represent the same likelihood or degree of exposure.

For example, an accountant who walks through a production area where trichloroethylene is used is potentially exposed, but the degree of contact (intensity) and duration are very limited. Another example is that residents in an area where some wells are contaminated are potentially exposed, but it is unknown if the well they used is contaminated. In this case, it is necessary to know the prevalence of contaminated wells or, ideally, whether the well serving the home was contaminated. Another example comprises measurements from a subset of workers with jobs where trichloroethylene is routinely used, and it is known that they are all likely to have been exposed. Even in areas with high exposures, some jobs may have only slight exposure, such as a

supervisor who stays in an office most of the day. Care must be taken to recognize the potential for misclassification in different exposure settings.

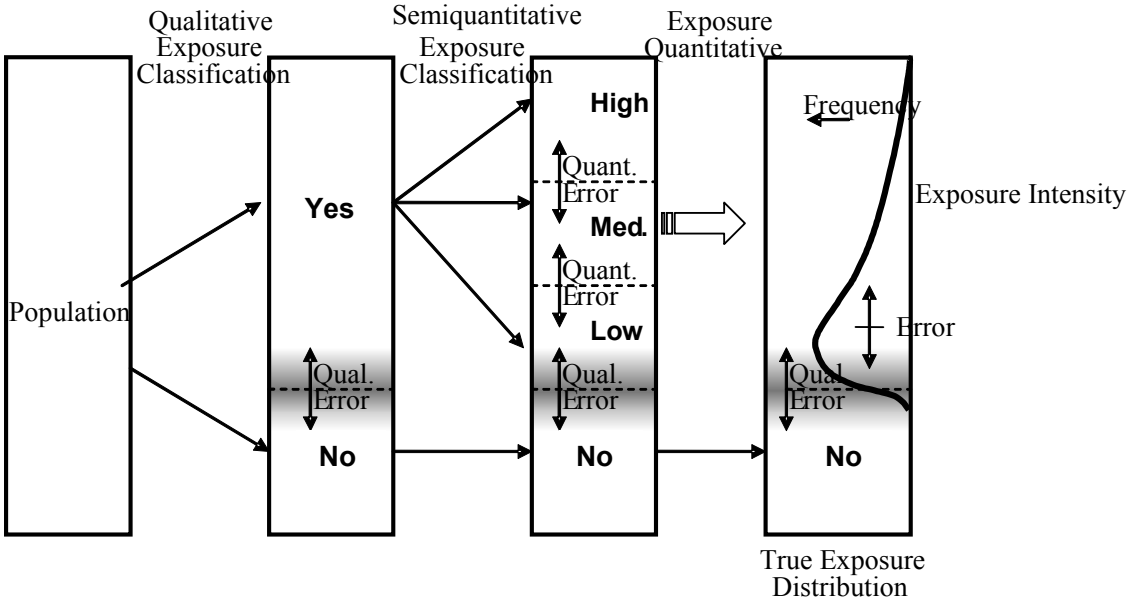

FIGURE 2-1 Exposure intensity classification approaches. Relative error rates are important for the utility of any of the approaches. There are two types of error: qualitative, for the presence of the agent; and quantitative, for assigning intensity of exposure.

Epidemiologic Approaches to Population Exposure Assessment

Exposure assessment uses a combination of approaches to answer two questions: (1) is the agent potentially present in the setting (workplace, community, home) and (2) if it is present, what were the intensity and duration of exposures (time profile of exposures)? For the first question, an agent (trichloroethylene) can be unequivocally shown to be present with no indication of the intensity of exposure, such as by identifying that degreasing operations were present and company purchasing records showing that large amounts of trichloroethylene were used. Given that trichloroethylene has been determined to be present, then we need to estimate the intensity of exposure. Intensity can be estimated from measurements, biological monitoring, and exposure modeling.

Six components of an exposure assessment determine the answers to the two questions above:

(1) Qualitative assessment
- Industry, community, neighborhood
- Use of trichloroethylene, prevalence of exposure
- Coexposures
- Confounders

(2) Exposure setting
- Location of exposures, area descriptors, or location of wells or contamination
- Relevant jobs, tasks, or personal activity factors associated with exposure
- Exposure controls (if any)

(3) Temporal data
- Data source(s), data quality
- Period covered
- Median duration of exposures
- Median latency

(4) Exposure quantification
- Measurement method (precision and accuracy define quality)
- Specificity (trichloroethylene measured, or nonspecific method for solvents)
- Quantity of data (extensive or limited)
- Temporal coverage of exposure data (current data only, or current and past data)

(5) Extrapolation methods
- Gaps in current exposure data, such as settings with low exposures, and estimating past periods (engineering-based model, or simplistic assumptions)
- Validation of past estimates (exposure data, or no validation)

(6) Dose metric
- Cumulative exposure, average exposure, duration in job, years exposed.

As in all exposure assessments, the researcher is limited by the data available and by the resources that can be applied to the task. These components define the quality of the estimates of exposure. If investigators have not given details on these aspects, then it is not possible to fully assess the quality of the data. It is not a requirement that all the data come from the study in question. Useful data often come from hygiene studies of the same industry or from community studies of similar settings. The goal is to form as complete a picture of the exposures as possible.

Information on Settings and Jobs

Information on workplace settings and jobs helps in the assessment of exposure. Factors to consider include description of workplace setting (size, layout, number of sources or tasks with emissions), specific sources of exposure (degreaser tanks [type, dimensions, solvents used, volume or time used, presence of covers, local-exhaust-ventilation controls]), and work tasks (use of degreasers, size of parts cleaned, manual cleaning with rag and bucket, hours per shift or per week cleaning).

The three primary types of degreasing and cleaning operations using solvents are listed in Table 2-1 with their approximate dates of use. Use of the vapor degreaser had the highest potential for exposure because vapors can escape from the degreaser, especially if poor work practices are used, such as early removal or too rapid removal that can carry concentrated solvent vapors and liquid out of the tank. Keeping the degreaser covered when not in use and careful operating procedures can minimize exposures. Dip tanks are the next important source of exposure. Hot dip tanks, where trichloroethylene is heated to close to its boiling point of 87ºC, are major sources of vapor that can be as important as vapor degreasers. Cold dip tanks have a lower exposure potential, but they have a large surface area and removal of the pieces can carry

solvent out. Small bench-top cleaning operations with a rag or brush and open bucket have the lowest exposure potential. Poor working techniques can distribute solvent across the bench top and to workers' skin and clothing. Less volatile solvents are generally used in manual cleaning activities. In combination with the vapor source, the size and ventilation of the workroom are the main determinants of exposure intensity.

TABLE 2-1 Years of Solvent Use in Industrial Degreasing and Cleaning Operations

Years	Vapor Degreasers	Cold Dip Tanks	Rag or Brush and Bucket on Bench Top
~1934-1954	Trichloroethylene (poorly controlled)	Stoddard solvent	Stoddard solvent (general use), alcohols (electronics shop), carbon tetrachloride (instrument shop).
~1955-1968	Trichloroethylene (poorly controlled, tightened in 1960s)	Trichloroethylene (replaced some Stoddard solvent)	Stoddard solvent, trichloroethylene (replaced some Stoddard solvent), perchloroethylene, 1,1,1-trichloroethane (replaced carbon tetrachloride, alcohols, ketones).
~1969-1978	Trichloroethylene, (better controlled)	Trichloroethylene, Stoddard solvent	Trichloroethylene, perchloroethylene, 1,1,1-trichloroethane, alcohols, ketones, Stoddard solvent.
~1979-1990s	1,1,1-Trichloroethane (replaced trichloroethylene)	1,1,1-Trichloroethane (replaced trichloroethylene), Stoddard solvent	1,1,1-Trichloroethane, perchloroethylene, alcohols, ketones, Stoddard solvent.

Source: Stewart and Dosemeci 2005.

Ranking by Semiquantitative Estimates of Exposure

Given an indication that some parts of the exposed population may have higher exposure than others, it may be possible to identify ranked subgroups by semiquantitative relative exposure differences, such as high and low or high, medium, and low. However, without knowing the relative toxicity or carcinogenicity of an agent, it is not possible to say what a "high" exposure is that also carries a high risk. Often, it is not possible to say how much more exposure one group has than another, but because of their frequency of contact or proximity to the emission source, a difference may be defined. If there is certainty that large differences exist, then comparing risks among exposure groups can provide some evidence that a dose-response relationship exists.

Semiquantitative classifications often have implicit assumptions that should be evaluated. For example, a job with task activities having direct contact with an agent is ranked higher than one that is in the same area but with indirect contact because it is assumed that direct contact provides more opportunity for intaking the agent. However, if the route of intake is respiratory and the source emissions are distributed broadly across a workplace, then there may be little difference between direct and indirect contact. This type of misclassification may not be detected where there is little detailed information about the setting or jobs associated with exposure.

Exposure Measurements

Methods used to characterize trichloroethylene are shown in Table 2-2. Initially, the methods were not specific for trichloroethylene and were rather imprecise. Thus, measurements may have overestimated the amount of trichloroethylene present in the environment by different amounts depending on what else was present in the exposure setting. After the early 1960s, specific and relatively precise methods were available. The charcoal tube collector with gas chromatograph analysis was available around 1974 and provides data with adequate duration, sensitivity, and selectivity. This method can define current and past exposure distributions of trichloroethylene among population subgroups and useful dose metrics can be defined. Unfortunately, these data were not available in most epidemiologic studies.

Nonselective or nonspecific methods, or the use of indirect "markers" or surrogates, cause problems in interpretation because only a portion of what they measure may be relevant to the risk, and the irrelevant portion can vary with the setting. For example, data on total chlorinated hydrocarbons, such as from a Davis halide meter, are useful for assessing the risk from trichloroethylene exposure if trichloroethylene vapor is the major component of the total chlorinated hydrocarbons or if the total chlorinated hydrocarbon mixture has a relatively fixed ratio of components. However, when trichloroethylene varies independently of the other chlorinated hydrocarbons in the mixture, then the measured total chlorinated hydrocarbons will provide only an illusion of relevant data and misclassification may occur. Detailed knowledge of the setting is critical for assessing the utility of the data.

A variety of dose metrics may be used to quantify or classify exposures for an epidemiologic study. They are summarized in Table 2-3 and include precise summaries of quantitative exposure, concentrations of biomarkers, cumulative exposure, and simple qualitative assessments of whether exposure occurred (yes or no). Each method has implicit assumptions and potential problems that lead to misclassification that need to be assessed.

Job-Exposure Matrices

Case-control studies are used to determine whether cases have significantly different exposures than controls. The ability of this study design to detect an increased risk of a specific exposure depends inter alia on the prevalence of the exposure in the base population. Case-control studies have been very useful for hypothesis generation where the goal is to provide evidence that broad categories of potential exposure are associated with increased risk in the general population. If a population has a common occupation or community exposure, then a case-control study may be useful for detecting an increased risk from that exposure. The best example of this is a nested case-control study, in which the cases and controls are drawn from a cohort with well-characterized exposures.

General population studies have special problems in evaluating exposure, because the subjects could have worked in any job or setting that is present within the population (Copeland et al. 1977; Nelson et al. 1994; McGuire et al. 1998; 't Mannetje et al. 2002). Jobs with high exposure are usually rare (low prevalence) in the general population. Only common jobs and settings have a high enough prevalence to be suitable for study. Asking subjects directly about their exposures to specific agents often underestimates exposures because workers do not know what chemicals they are using, or they do not understand what is meant by exposure.

TABLE 2-2 Methods for Measuring Trichloroethylene

Method	Principle	Years Used	Limit of Detection	Specificity
Absorption in ethanol	Bubbler with ethanol absorbs trichloroethylene (<30 min), only area samples; several analysis methods:			
	combustion, titrate total chlorine;	1947-1954	~20 mg/m^3	Nonspecific, ±30%
	add pyridine, colorimetric assay;	1954-1962	~20 mg/m^3	Nonspecific, ±30%
	inject in gas chromatograph for analysis.	1962-1973	~1 mg/m^3	Specific, ±20%
Davis halide meter	Chlorinated hydrocarbons combusted; real-time data on breakdown products.	1954-1978	~20 mg/m^3	Nonspecific, ±30%
Dräger tube	Air drawn through special tube (<1 min), chlorinated hydrocarbons break down and react with dye, read the color.	1959-1974	~20 mg/m^3	Nonspecific, ±50%
Gas pipette	300 mL of air collected (<1 min), then gas chromatograph analysis.	1963-1973	~1 mg/m^3	Specific, ±20%
Charcoal tube	Task or full-shift personal samples; integrated sample collected on charcoal tube, then gas chromatograph analysis.	1974-1984 1885-1989	0.8 mg/m^3 0.3 mg/m^3	Specific, ±20%

Source: Raaschou-Nielsen et al. 2002.

TABLE 2-3 Plausible Exposure Metrics

Metric	Calculation	Implicit Toxicologic Assumptions	Applications and Possible Problems
Cumulative exposure (CE)	CE = Σ (exposure × years) for all jobs, tasks, or locations with exposure	Linear accumulation of risk; irreversible. Risk from long, low exposure equals short, intense exposures.	Common cancer metric Problems with: Nonlinear effects Reversible effects Effects from peaks.
Average exposure (e.g., during all jobs)	Avg = CE/Σ (yr)	Duration is irrelevant to risk, or all subjects had similar durations.	Good for reversible effects. Problems with: Wide differences in duration Effects from peaks.
Duration of exposure (from all sources)	Dur = Σ (yr)	Accumulation of risk; all subjects had similar average intensities.	Good for cumulative risk where enough subjects have had sufficient intensity to increase risk with duration. Problems with: Wide average intensity differences

TABLE 2-3 *Continued*

Metric	Calculation	Implicit Toxicologic Assumptions	Applications and Possible Problems
Peaks (short duration, $\approx T$, exposures with concentrations $> X$ threshold)	Frequency per time Probability of exposure $> X$ Highest peak	Peaks with duration $< T$ or intensity $< X$ do not increase risk. Higher peaks contribute to risk.	Good for threshold effects, and risks with peaks. Problems with: No threshold Wrong T or X setting Few subjects had peaks with T duration Few subjects had peaks $> X$ threshold.
Job duration	Years in job	Accumulation of risk. Only one job contributes to risk and enough subjects in job have had sufficient intensity and duration to increase risk.	Problems with: Reversible effects Many jobs with exposure Few have had sufficient intensity to increase overall risk.
Longest job	Job with most years	Only one job contributes to risk and enough subjects in job have had sufficient intensity and duration to increase risk. No risk from jobs with short exposure.	Problems with: Many jobs with exposure Too few subjects with sufficient intensity and duration to increase overall risk.
Ever worked in job	Job title	Only one job contributes to risk. Any type of exposure will increase risk.	Problems with: Many jobs with exposure Too few subjects with sufficient intensity and duration to increase overall risk.
Exposed (yes/no)	Ever any contact with agent	Enough of exposed have had sufficient intensity and duration to increase risk.	Problems with: Too few subjects with sufficient intensity and duration to increase overall risk.

Considerable care is needed in constructing questionnaires to obtain useful data (Stewart et al. 1998). Increased risk is likely to be undetectable unless the exposure is common and high. This problem is analogous to the problem of studying a rare disease in a cohort. Simulation studies have been done to define the relationship between the risk and prevalence of exposure and incidence of disease (Thomas 1987).

Several exposure assessment techniques have been developed for general population-based case-control studies, including job-exposure matrices and occupational survey questionnaires. These techniques are frequently used together. General questionnaires are limited in what they can ask about occupation and specific exposures. Most occupations use jargon that varies across industries and companies. As a result, it is generally necessary to translate job titles and industries reported by subjects on questionnaires into standardized titles, such as those developed by the International Labour Office (Quinn et al. 2001). Various investigators have developed job exposure matrices to collect information about typical occupational exposures in common industries in a city or country. The more a job exposure matrix is tailored for a specific area or industry, the less misclassification it will have. The utility

of a broad job exposure matrix depends on the uniformity of exposures for a job title within an industrial sector. Unfortunately, it is also common for exposures to be heterogeneous and variable, both qualitatively and quantitatively, across companies and time periods. Even a specific company's facility can have large variations among workers and over time for workers with the same job title (Quinn et al. 2001). Thus, the misclassification in job exposure matrices may be quite large. Job exposure matrices work best when exposures are common and intense.

Job exposure matrices are also developed in some cohort studies where data are highly detailed (Smith et al. 1995; Tielemans et al. 1999; Le Moual et al. 2000; Quinn et al. 2001). These matrices are specific for the cohorts being studied. Extensive record reviews, long-term worker interviews, and data analysis are used to characterize the settings, job titles, task activities, materials used, production activities, and history of changes over the years. These data collection and analysis activities are highly labor intensive and costly, so few have been conducted. However, where they have been done they can produce the highest-quality exposure assignments depending on the limitations of the available data and resources.

Classifications of Exposure into Binary (Ever-Never) Scales

Exposures assigned to workers may also be analyzed according to whether they ever or never worked in a particular job classification or according to the longest held job, because it is common for an individual to have worked in several jobs or industries. Duration of work in an industry is often used as a surrogate of exposure, with the implicit assumption that exposure occurred every year of work and at the same average intensity each year. This will likely produce meaningful classification only if the exposures were very intense in one job or segment of an industry. For example, the high risks of lung cancer from exposure to coke-oven emissions were undetectable within the whole population of a steel plant in Pittsburgh but were readily seen among coke-oven workers who had high exposures, especially the topside workers (Lloyd and Ciocco 1969; Lloyd 1971).

Qualitative errors in assignments of exposure may be minimal when there are clear data on the presence or absence of an agent in a location, worksite, or community. However, epidemiologists have tended to err toward identifying as exposed any individual with even minimal potential exposure. The problem with this classification system is a large dilution of actual risk because there are often a large number of individuals at the lowest end of the true intensity range (as shown in Figure 2-1). These intensities of exposure are so low that the increase in risk is virtually undetectable. When that happens, even the observed risk for exposure to a known carcinogen, such as asbestos, is only slightly elevated. This was seen in studies of railroad workers and workers making man-made mineral fiber products (Garshick et al. 1987; Marsh et al. 2001). Additionally, when studies of different populations are compared, "exposed" is often used as if it implies the same dose—that is, the same distributions of exposures and durations across the populations. Although this may be true, it is seldom verified (that may not be possible). Clearly, it is inappropriate to equate a population with only low exposures to one in which a significant fraction of the population had high exposures. Assigning "exposed" to a population is implicitly a quantitative assignment because it means exposure is not zero, but it does not also imply that increased risks are detectable.

Biomarkers

In some studies, exposure may be estimated by the concentration of a biomarker. An exposure biomarker is an internal substance in a readily accessible biological medium—most commonly breath, blood, or urine—that can be used to indicate exposure. There are four broad types of biomarkers that have different applications: exposure, response, susceptibility, and disease. Biomarkers may be highly specific, such as saliva cotinine for cigarette smoke, or nonspecific, such as urinary 1-hydroxypyrene for polycyclic aromatic hydrocarbons, which are found to some degree in all combustion emissions. Some biomarkers may be used for more than one type of application; for example, a decrease in red-blood-cell cholinesterase caused by organophosphate pesticides may be an indication of exposure or of a health outcome. Depending on the setting, the biomarker may be useful, prone to misclassification, or difficult to interpret. The utility of a biomarker depends on its selectivity and the exposure situation. A nonselective biomarker of exposure, such as total urinary chlorinated hydrocarbons, may be very useful if nothing but trichloroethylene is present in the workplace. However, in settings with mixed exposures it may be difficult to interpret the concentration of a nonspecific biomarker. It is rare for a biomarker alone to define exposure in an epidemiologic study; supplementary data are nearly always needed.

Exposure Metric

An exposure metric is a summary number or category that defines exposure in an epidemiologic analysis. The range of exposure metrics generally available for epidemiologic studies is summarized in Table 2-3. None of them is without problems, and they are not equally useful. If one's goal is to define the exposure-risk relationship, then the closer one is to a personal dose over the duration of exposure, the more precisely the relationship can be defined because individuals become diseased because of their personal risk factors. The level of misclassification increases as one moves down Table 2-3. Recall that misclassification applies to both the qualitative and quantitative measures of exposure.

Exposure Assignment Errors

Exposure to chemicals is a personal attribute that has three important dimensions: composition, concentration, and duration. These define a time course at the point of entry, which can be summarized by a dose metric, such as cumulative exposure (average intensity time duration). Ways that these features of exposures may be defined were identified above, as well as common problems defining one or more of these dimensions for individuals in a study population. The primary source of errors is that it is rare that an individual's personal exposure can be estimated or extrapolated. Commonly, one or more personal characteristics, such as home address, job title, residence, or occupational history is used to assign an exposure category to everyone with those characteristics. The source(s) and level of detail of the data for these characteristics is important and usually one of the major limitations of the estimates. Below some of the most common sources of error in exposure for epidemiologic studies are discussed.

Qualitative Exposure Assignments

The determination of the presence or absence of a particular chemical in a subject's immediate environment is the first step in any exposure assessment. The rationale used for the determination is a central factor controlling the accuracy of the assignment. For example, the rationale "dry cleaning attendants are potentially exposed to trichloroethylene" is true for some dry cleaners before 1980 in the United States, but not all and currently most cleaners do not use it. Alternatively, "dry cleaning attendants in cleaners with records showing purchases of cleaning fluid containing trichloroethylene are exposed" is more likely to be true. The more general the job classification and broader industry grouping, the greater the misclassification will be. For example, as company size increases the proportion of workers with trichloroethylene exposure decreases. Many workers are exposed in small companies whereas only a small fraction will be exposed in large companies. Investigation of the distribution of the workers across jobs and industries can determine how much misclassification there is, but this has not been done for the occupations studied in the available papers. The other common misclassification problem is the potential for exposure is too low, so that even though exposure is not zero, the probability of an effect is not distinguishable from zero (e.g., large residential areas where only a few houses may have drawn water from a contaminated well; an accountant who occasionally passes through a workshop area where trichloroethylene is used).

When assigning potential exposure, it is implicitly assume that the exposure may be high enough to affect risk, which often is not true. Assessing the risks of large numbers of individuals with low or no exposure will limit the power of a study to observe increased risk. One unfortunate limitation of environmental studies is that the residents of an area or the workers rarely know the specific chemicals that they come in contact with or the materials they are using. Similarly, individual home owners or area residents rarely know the chemicals they are exposed to in water or ambient air, and do not know the local sources of chemical exposures. Consequently, the researcher must be able to develop a rationale to link the subject to the exposures.

Quantitative Exposure Assignments

Given that significant exposures probably occurred in an industry or residential area, the more precisely that questions about the nature of the exposures can be answered for the subjects in a study, the less misclassification there will be. What is the material of interest? Where is/was it used, which tasks were performed, and what quantity is/was used? Are there data indicating the exposure intensities? How specific and precise were the measurement methods? How well can work areas and/or job tasks be linked to individuals (e.g., through job titles and/or work histories)? Those questions must be answered to make quantitative estimates. When there has been a wide range in exposures, a study can overcome some random misclassification. A combination of descriptive and measurement data can be used to quantify exposure. These assignments have some misclassification error. The precision and accuracy of the information usually decreases the further into the past one tries to make an assessment.

Collecting data by interviewing long-term workers or area residents is a common way to obtain descriptive exposure data. These data are subject to all of the usual limitations of individual memories and biases in recall. Given detailed data on materials used, the place it is

used, applications, and worker activities, industrial hygienists can make semi-quantitative estimates based on models using first principles of ventilation and chemical behavior and general data on exposure associated with similar settings. This approach can provide order of magnitude estimates that are useful for distinguishing groups of workers with large differences in exposure (i.e., larger than a factor of two). Smaller differences usually cannot be reliably distinguished.

Temporal Differences in Exposure

Company records with job titles and work locations can provide data with a high degree of accuracy and precision. This is important for defining the duration of exposure, when it is clearly linked with jobs or work locations. Clearly if there is uncertainty about where exposures occurred, there will be corresponding uncertainty about the duration. Similarly, residence time at a particular location can be used as a measure of exposure duration. One of the most common semi-quantitative environmental dose metrics is duration of exposure. This metric is based on an implicit assumption that exposure intensities were all approximately the same in an area or job, which is rarely the case. Therefore, the misclassification may be large and no association of risk with duration may be detected. It is sometimes assumed that the relationship between duration and risk is a determination of the dose-response relationship, but that is a weak test for the relationship. If supplementary data can show that exposures were clearly present and may have been elevated, then misclassification may be less of a problem. These issues were present for several of the studies reviewed by the committee.

In general, nearly all of the studies reviewed had weak classification of exposure, particularly exposure intensity. The analyses of the literature were limited in their assessment of the exposure for the meta-analyses. A more sophisticated and knowledgeable analysis of the exposures in the available studies is needed. In Chapter 3 and Appendix D, epidemiologic studies of kidney cancer are evaluated to illustrate the methodology by which this might be done; the evaluation showed that some studies were more informative and others less informative than the authors concluded.

COMBINING AND EVALUATING EPIDEMIOLOGIC DATA

Strategies for Including Studies in the Risk Assessment for Exposure to Trichloroethylene and Cancer

A full review of the literature should identify all published studies in which there was a possibility that trichloroethylene was investigated, even though results per se may not have been reported. It is important to appreciate the number of studies in which associations could have been identified so as to understand the universe of the relevant literature. In particular, there are case-control studies that make use of job exposure matrices or other modalities for assessing exposure that may have included trichloroethylene but did not publish results because of negative findings. For example, the analyses by Parent et al. (2000) of renal cell cancer and Goldberg et al. (2001) of colon cancer, which were based on a multisite case-control study designed by Siemiatycki (1991), assessed risks for occupational exposure to trichloroethylene

but the specific findings were not published because they did not meet the threshold for the magnitude of association to be included in the paper.

There need to be clear a priori guidelines about what types of studies should be included in a risk assessment. The committee concurs with EPA's (2005a) *Guidelines for Carcinogen Risk Assessment*, which stipulates that cohort and case-control studies are the main types of studies that can be used to draw conclusions about associations and causation. The guidelines suggested that "all studies that are considered to be of *acceptable* quality … should be considered in assessing the totality of the human evidence" (EPA 2005a, p. 2-4, emphasis added), but otherwise no specific criteria for inclusion or exclusion were presented. What constitutes a study of acceptable quality is a difficult question and there likely is no clear-cut answer. Epidemiologists may agree to some extent about classifying studies that are of superior or inferior quality, but most studies lie somewhere in between. Thus, what is an acceptable study is a question that may be framed better by evaluating the different methodological attributes of studies and, possibly, incorporating them in a quantitative analysis (meta-regression).

For the sake of transparency of the risk assessment, tables and figures that explicitly summarize the essential design characteristics and results of studies must be included in documentation of the risk assessment. For the purposes of illustration, the committee has used one possible mode of presentation borrowed from the Institute of Medicine's Gulf War and Health study series (IOM 2003; see Chapter 3). An ACCESS database was developed for that project to classify various attributes of the studies, and this may be a useful method for summarizing methodological attributes of studies as well as results. Other formulations are possible.

The committee believes studies should be excluded if there is little probability of exposure to trichloroethylene; inclusion of such studies means that any risk analysis, qualitative or quantitative, would lead to incorrect inferences. Moreover, it is important to attempt to group studies by level of presumptive exposure ("meta-exposure"). The chapters that follow will expand on these issues, especially as related to kidney cancer (Chapter 3).

Should a quantitative summary of the data be conducted (meta-analysis) according to whether subjects in the study were "ever exposed," the committee suggests attempting to group studies according to some index of exposure, in a stratified or other type of analysis. These recommendations are consistent with EPA's guidelines:

> For epidemiologic data to be useful in determining whether there is an association between health effects and exposure to an agent, there should be adequate characterization of exposure information. In general, greater weight should be given to studies with more precise and specific exposure estimates.
>
> Questions to address about exposure are: What can one reliably conclude about the exposure parameters including (but not limited to) the level, duration, route, and frequency of exposure of individuals in one population as compared with another? How sensitive are study results to uncertainties in these parameters? (EPA 2005a, p. 2-6).

Again, these issues are discussed in more detail in other chapters, especially with regard to the association between kidney cancer and exposure to trichloroethylene.

Methods to Summarize the Literature

A number of methods have been used to present relevant results from the epidemiologic literature. The narrative review is a verbose method in paragraph style that usually describes and summarizes the methods and principal findings for each study; at the end, some form of summary and conclusions are provided. Often, studies are classified as being positive or negative and "ballot counting" (the number of studies with "positive findings" is divided by the total number of studies in which the association was evaluated) is used as a criterion for making decisions about statistical and causal associations. Ballot counting can be misleading if studies are heterogeneous on levels of meta-exposure. (Should ballot counting be used, then appropriate statistical methods need to be used to calculate *P* values for the proportion of positive studies.) If one takes the reasonable point of view that results from studies should be viewed as meta-data, then a more reasonable approach is to provide detailed tables and figures that summarize the results, especially as related to some index of meta-exposure (see examples in Chapter 3). The narrative can be used to discuss the strengths and limitations of the studies and the tables.

General Principles of Meta-Analysis

Meta-analysis, frequently referred to as the study of studies, involves a statistical analysis of the findings from several studies. It provides a quantitative means to combine and contrast results from different studies to identify patterns and sources of disagreement among the results (Rothman and Greenland 1998). Epidemiologic studies are generally highly variable with respect to their design, quality, and level of exposure to the hazard under investigation. Because of these differences, the results from epidemiologic studies can be highly variable or, in statistical terms, "heterogeneous." Meta-analysis has been applied to epidemiologic studies to provide a quantitative summary of the evidence. Such an analysis may be accomplished in several ways. The simplest method, sometimes referred to as ballot counting, is to count the number of studies that are positive or negative. This method is generally not informative, because it gives equal weight to each study regardless of study size or quality. A somewhat more refined method is to compute an average of the findings from the studies, which may be weighted by the inverse of the variance of the individual studies. This approach does take into account the size of the studies (which is related to the variance) but does not consider other differences related to the quality and designs of the studies or their exposure assessments. By today's standards for meta-analysis, simple ballot counting or even weighted averaging of findings generally is not considered an adequate analysis. Statistical methods for meta-analysis are now available to assess the extent of heterogeneity among studies, and for fitting of random effects models to account for heterogeneity when it exists (DerSimonium and Laird 1986). This approach presumes that the main source of variation is statistical, but systematic differences in population exposures and methods for assessing them can also be important. However, in many instances epidemiologic data are too variable to justify combining them no matter how sophisticated the statistical methods. Thus, some authors have suggested that the primary goal of an epidemiologic meta-analysis is more often to identify the source of heterogeneity in the study findings than to produce an overall or summary estimate of the effect (Greenland 1987).

A large number of issues arise when performing a meta-analysis, particularly when such analyses are based on observational data (e.g., epidemiologic) as opposed to experimental data

(e.g., clinical trials). Many of the decisions are largely subjective—for example, which studies to include (e.g., cohort, case-control), which results to use from each study (e.g., lagged or unlagged relative risks), and how to treat studies of questionable quality (e.g., eliminating them, using quality scoring). Such decisions are largely subjective and should be made carefully because they can affect the outcome of the meta-analysis. In addition, limited attention is usually given to problems arising from the wide variation in the quality and level of detail in exposure assessments for the studies (see discussion of the Wartenberg et al. [2000] analysis below). As a result, even though the goal is to evaluate the relationship between exposure and disease, only the disease dimension is critiqued with any sophistication.

Another common issue in meta-analysis, sometimes called the "file drawer" problem or publication bias, refers to the fact that positive studies are more likely to be published than negative ones. Conversely, the possibility also exists that some positive epidemiologic studies might not be published. Statistical methods have been developed to evaluate whether there is evidence for publication bias. These methods rely on constructing a plot of the findings, observed relative risk versus the variance of each of the studies, which is commonly referred to as a funnel plot (Light and Pillemer 1984). One generally expects that the negative studies that do not get published are small and have large variance. Implicit in this type of analysis is the assumption that the effects of differences in methods and exposure assessments are random and small. Thus, if a publication bias exists, one would expect to see more small studies showing a positive finding than large ones. However, this approach is not a powerful method for detecting publication bias, and there is no way to check the basic assumptions.

Meta-analysis of epidemiologic data remains somewhat controversial, despite advances in the methodology. Some epidemiologists have questioned whether meta-analysis is useful for summarizing epidemiologic data given the inherent problems of combining epidemiologic data (e.g., Shapiro 1994). Other epidemiologists have defended meta-analytic methods, particularly when they are properly applied (e.g., Petitti 1994). Despite these controversies, most epidemiologists have come to view meta-analytic methods as a useful, albeit imperfect, tool for performing a quantitative summary of the epidemiologic evidence. Following is a discussion of some specific issues for performing a meta-analysis of the epidemiologic data on trichloroethylene and cancer.

Specific Meta-Analysis Issues for Trichloroethylene

The committee reviewed two meta-analyses that were performed to examine the association between trichloroethylene exposure and the risk of cancer. The first analysis, by Wartenberg et al. (2000), was heavily relied on in the EPA (2001b) draft risk assessment on trichloroethylene. The second, by Kelsh et al. (2005), was presented to the committee at a meeting on June 9, 2005. Each analysis is discussed below in light of some of the general principles discussed above and in light of some criticisms pertaining to the published analysis by Wartenberg et al. (2000).

Analysis by Wartenberg et al. (2000)

The review by Wartenberg et al. (2000) presents estimates of the relative risk for kidney and renal cell cancers, liver and biliary cancer, non-Hodgkin's lymphoma, Hodgkin's disease, cervical cancer, and pancreatic cancer. The analyses were stratified into three tiers on the basis of the authors' subjective judgment of the quality of the exposure data in the studies, with the first tier having the highest quality. The Tier I studies had direct information on exposures (biomarkers, job exposure matrices, job histories), Tier II studies were based on job title, and Tier III studies were of dry cleaner and laundry workers.

A weighted average of the relative risks reported in the cohort studies was estimated where the weights were the inverse of the variance of each study. This analysis provided evidence supporting an association in the Tier I studies for exposure to trichloroethylene and increased risk of kidney cancer relative risk [RR] = 1.7, 95% confidence interval [CI] = 1.1-2.7), liver cancer (RR = 1.9, 95% CI = 1.0-3.4), and non-Hodgkin's lymphoma (RR = 1.5, 95% CI = 0.9-2.3). To a lesser extent, the analysis provided evidence for an association between exposure to trichloroethylene and cervical cancer, Hodgkin's disease, and multiple myeloma.

Letters to the editor (Borak et al. 2000; Boice and McLaughlin 2001; Rhomberg 2002) have criticized this quantitative analysis. The common criticism relates to including the study by Henschler et al. (1995) in the analysis. There were several methodological concerns about that study (see discussion in Chapter 3). One of the main objections was that the cases were originally identified in a cluster investigation, which does have relevance in interpreting the study. Clusters do occur by chance and, if epidemiologic studies are performed in areas or industries with known clusters, they clearly may be biased toward observing an effect. On the other hand, many of what are considered to be known occupational carcinogens (e.g., vinyl chloride, bis[chloromethyl]ether) were originally identified from clusters and subsequent formal studies of the same population confirmed that the cancers were truly in excess. Excluding these types of studies would clearly introduce a negative bias into a meta-analysis. However, the variance of the estimate of the relative risks, as reported by Henschler et al., is underestimated, as it does not account for the fact that the study was based on a non-random sample (cluster); in principle, its formal use in a meta-analysis would need to make use of a corrected (inflated) variance. The Henschler et al. study also was unusual in that it reported an extremely large relative risk of kidney cancer (RR = 8.0, CI = 3.4-18.6). Although a corrected variance ideally should be used, the committee is unaware of methods to adjust the variance of a study that is based on a cluster.

Some reviewers have minimized the importance of the Henschler et al. study by referring to it as an "outlier." However, there is evidence that the exposure concentrations of trichloroethylene might have been higher at the facility studied by Henschler et al. than in other studies (see Chapter 3), providing a plausible explanation for the unusually high relative risks for kidney cancer that were observed. Other methodological concerns with the study have been raised by several authors (see Chapter 3). One way to assess these issues formally in the meta-analysis is to conduct sensitivity analyses by including and excluding the study to determine whether it strongly influences the results of the meta-analysis.

One problem with meta-analysis is that each study is analyzed in isolation, such as the one by Henschler et al. (1995). However, a series of studies have been done on the general population in the geographic area where the study was conducted, which appears to have a segment with substantial exposures (Vamvakas et al. 1998; Brauch et al. 1999, 2004; Pesch et al.

2000a; Bruning et al. 2003). Those studies have investigated different segments of the population and different outcomes, and they all found evidence of kidney cancer. If the Henschler et al. (1995) study is considered in that context, then it is consistent with the nature of the population and the level of occupational exposures inherent in the working population.

The summary relative risk estimates presented by Wartenberg et al. (2000) did not include case-control studies and generally emphasized the Tier I cohort studies. It was suggested that case-control studies are inferior to cohort studies because they generally lack detailed information on exposures. However, this is not always the case (see previous discussion in this chapter) and certainly is not the case for nested case-control studies, such as the study by Greenland et al. (1994) that was excluded from the Wartenberg et al. analysis. Methods are available for including case-control studies with cohort studies in a meta-analysis (see Greenland 1987).

Wartenberg et al.(2000) did not include any formal statistical analysis of the studies for heterogeneity. Testing for and evaluating heterogeneity is standard practice in meta-analysis. It appears that the findings for kidney cancer may not have been homogeneous given the unusually large effect reported by Henschler et al. (1995). Wartenberg et al. (2000, p. 174) recognized the need for further work and recommended that a meta-analysis be conducted that would "try to isolate the factors that help explain the observed risks, as well as to better quantify the risk. One would have to focus carefully on the possible heterogeneity among studies, carefully considering which groups of studies to combine." In addition, one could conduct meta-regression whereby meta-characteristics of studies are used to help explain the observed heterogeneity (one important factor would be levels of meta-exposure). Finally, a much more detailed assessment could be made of the exposure data used in each of the studies. For example, studies with very high exposures provide information on a different part of the exposure-risk curve than those with lower exposures.

Analysis by Kelsh et al. (2005)

Kelsh et al. (2005) performed a meta-analysis of the epidemiologic studies of trichloroethylene, and this information was presented to the committee at a meeting on June 9, 2005. (The study reportedly has been submitted for publication.) The review included several studies that were published after Wartenberg et al. performed their analysis, including cohort studies by Hansen et al. (2001) and Raaschou Nielsen et al. (2003) and a case-control study of kidney cancer by Pesch et al. (2000a). Several studies used by Wartenberg et al. (2000) were excluded from the meta-analysis. In part, these omissions are explained by Kelsh et al.'s exclusion criteria, which eliminated proportional-mortality-ratio, community, and cross-sectional studies. Excluding proportional-mortality-ratio and cross-sectional studies might be warranted based on concerns about the quality of these types of studies. However, well-designed proportional-mortality-ratio studies can yield results of similar quality to full cohort studies under certain conditions (Monson 1974; Wong and Decoufle 1982). It is difficult to justify eliminating community studies, because they provide the only information on the effects of exposures from water contamination with trichloroethylene, unless they are purely ecologic. The community studies have more limited exposure assessments but, under some circumstances, they can provide useful information about risks of exposures in the community.

Some of the other exclusions made by Kelsh et al. are more difficult to explain. For example, the following cohort studies that were included by Wartenberg et al. (2000) were not used in the analysis by Kelsh et al.: Wong and Morgan (1990), Spirtas et al. (1991), and Boice et al. (1999). They appear to be relatively high-quality studies that should have been included. However, they had poor quality or limited exposure data.

Similar to Wartenberg et al., Kelsh et al. categorized the cohort studies into two groups based on quality concerns. Group I studies had clearly identified exposures to trichloroethylene from biomonitoring, industrial hygiene, chemical inventories, or job exposure matrices. Group II studies either had limited documentation of exposures to trichloroethylene or had "quality of data limitations." There was generally good correspondence between those cohort studies that were classified as Tier I and II by Wartenberg et al. and Group I and II by Kelsh et al., with one notable difference—the study by Henschler et al. that Wartenberg et al. classified as being of the highest quality (Tier I) and that Kelsh et al. classified as being of lower quality (Group II). This difference points to the subjective nature of these qualitative rankings of studies.

The statistical methods used in the analysis by Kelsh et al. are more consistent with modern methods for meta-analysis than those used by Wartenberg et al. Random effects models were fitted and tests were performed to evaluate the heterogeneity of the meta-results. Sensitivity analyses were conducted in which one study at a time was deleted from the meta-analysis. Kelsh et al. analyzed case-control studies separately from cohort studies in their analysis, which is similar to what Wartenberg et al. did. As noted previously, case-control and cohort studies may and should, if possible, be combined in a meta-analysis, and the basis for assignments of exposure needs to be carefully assessed to determine the level of misclassification by qualitative and quantitative criteria (as discussed earlier).

Kelsh et al. reported an elevated and statistically significant meta-analysis relative risk for kidney cancer (RR = 1.29, 95% CI = 1.06-1.57) among the Group I studies, which was not heterogeneous ($P = 0.90$). The analysis of the Group II and case-control studies also showed elevated risks, but the findings were heterogeneous and highly dependent on including "outlier" studies. The authors suggested that the positive findings for kidney cancer might be explained by smoking or more intensive health monitoring in worker populations, particularly in U.S. workers. Both explanations appear unlikely to the committee. Smoking is a relatively weak risk factor for kidney cancer with relative risk estimates for smokers being generally less than 2 (IARC 2004), and it seems unlikely that the industry studies are likely to have had special screening programs for kidney cancer for trichloroethylene-exposed workers.

Kelsh et al. also reported a statistically significant increase in risk in their meta-analyses for liver cancer (RR = 1.32, 95% CI = 1.05-1.66) and non-Hodgkin's lymphoma (results not reported but the 95% CI presented graphically clearly excludes unity). In both cases, the authors suggested that there was heterogeneity of these findings with European studies showing higher risks of liver cancer and non-Hodgkin's lymphoma, even though the test for heterogeneity of these findings was not statistically significant (liver, $P = 0.34$; non-Hodgkin's lymphoma, $P = 0.18$). The authors also emphasized the lack of evidence for an exposure-response relationship for these end points and suggested that was a reason for rejecting a causal association. The committee disagrees with this suggestion—lack of an exposure-response relationship is not convincing evidence against a causal interpretation because these studies generally lacked the information for estimating amount of exposure (Stayner et al. 2003). When considered across studies, there is a trend for increasing risk where there is evidence of higher exposure.

Use of Bradford-Hill's Guidelines to Assess Causality

Reviews of epidemiologic data may be qualitative or quantitative. Qualitative reviews have frequently relied on interpreting findings with the set of guidelines first proposed by Sir Bradford Hill (1965). These are often referred to as "criteria" and the committee was specifically asked to comment on the use of these "criteria." In fact, Hill referred to them as "viewpoints" and he emphasized that none of them was necessary or sufficient. Thus, it is a mistake to view the "Hill criteria" as a checklist that must be completed before causality is determined.

These guidelines generally include consideration of the (1) strength of the association, (2) evidence for an exposure-response relationship, (3) consistency of the findings between studies, (4) biological plausibility of the hypothesis, (5) temporality of the exposure (does it precede disease), (6) specificity of the exposure and disease relationship (is exposure associated with a specific disease), (7) support from analogy, and (8) support from experiments. These guidelines are extremely useful, but most epidemiologists do not consider any one of them to be a necessary condition for causality except for the one stating that exposure must precede the onset of disease.

Hill's guidelines do not directly address problems with the quality of the exposure assignments, but they are implicit in many of his viewpoints. It is not clear why the "specificity of the exposure and disease relationship" is important—single agents can cause more than one outcome (e.g., cigarette smoking), and an outcome can be caused by more than one agent (e.g., smoking).

Exposure-Response Analysis

Information on exposure-response relationships derived from epidemiologic investigations play a critical role in the hazard identification and dose-response evaluation elements of risk assessment. Strong evidence for an exposure-response relationship is one of the key pieces of evidence that epidemiologists use to make inferences about causality. It is one of the elements of the Hill guidelines for judging causality that have been incorporated into the new EPA (2005a) cancer guidelines for hazard identification.

The absence of evidence for an exposure-response relationship does not often provide a convincing argument against causality. For one, if the exposure estimates are inaccurate or imprecise then it is well recognized that there may a bias in the exposure-response relationship toward the null, and this bias might even eliminate the relationship under certain conditions (Armstrong 1990; Dosemeci et al. 1990; Steenland et al. 1996, 2000). Studies may be negative because there are too few subjects with sufficient exposure to increase overall risk or because controls have unrecognized exposures. Furthermore, there are numerous examples in occupational epidemiology where exposure-response relationships observed in a study flatten or even decrease at the highest exposures (Stayner et al. 2003). The reasons for this are unclear; however, they might be explained by biases in the studies (e.g., the healthy worker survivor effect), by biologic factors (e.g., a saturation of key enzyme pathways at high concentrations), or by misclassification in the highest exposure assignments, which can only be misclassified downward (some highly exposed subjects are assigned to medium exposures).

Hertz-Piccioto (1995) has suggested that epidemiologic studies that are suitable for quantitative risk assessment should (1) provide evidence for a moderate-to-strong exposure-

response relationship, (2) have strong biases and confounding ruled out, and (3) have exposures linked to individuals. These criteria are perhaps somewhat overly restrictive, and very few epidemiologic studies will meet them all. In practice, it may be helpful to use epidemiologic studies for quantitative risk assessment even when they do not meet all the criteria if for no other reason than to provide a test of the reasonableness of risk estimates derived from toxicologic or other epidemiologic investigations (for testing the validity of other risk assessment models).

As noted earlier in this chapter, the most common limitation of epidemiologic data for quantifying risk is the availability of high-quality exposure data to construct exposure-response models. Hardly any epidemiologic studies have actual measurements of individual exposures for each study subject over the entire study time period with the exception of studies of workers in the nuclear power industry. More commonly in occupational studies, as discussed above, a job exposure matrix may be developed to estimate each individual's exposure based on job title, work location, or industry. In the absence of measurements of personal exposure or exposure estimates of groups based on job characteristics, residence location, or water supply (see Siemiatycki et al. 1981; Siemiatycki 1991), only broad classifications generally are available for conducting exposure-response modeling for quantitative risk assessment purposes with epidemiologic data. Unfortunately, these types of exposure data were rarely available in the epidemiologic studies of trichloroethylene.

Surrogates for quantitative estimates of exposure such as duration of exposure or exposure category (e.g., high, medium, low) are available for some of the trichloroethylene studies. Crude risk assessment models may be derived from these studies if some assumptions are made about the average exposures of individuals in the study groups. This approach is highly prone to error and should be used only to determine whether there is evidence to suggest a hazard. Where there is considerable misclassification of intensity of exposure, there may be no evidence of an exposure gradient even when there is good evidence of an increased risk for ever-exposed versus never-exposed subjects. If an exposure-response relationship is seen, it is likely that the slope is underestimated. If preventive action is needed, surrogate exposure information can be used when other more appropriate exposure-response information is unavailable.

Occasionally, it may be possible to use biologic measures (e.g., urinary biomarkers) to estimate the exposures for study subjects (see discussion earlier in this chapter). These measurements have the advantage of integrating personal factors, such as metabolism of the compound, and thus may come closer to estimating the true dose resulting from an exposure. Because metabolic enzymes vary across individuals, if the carcinogenic agent is a metabolite, then use of the biomarker will increase the variability in the exposure assignments for the subjects in each exposure group. In the case of trichloroethylene, several studies from Scandinavia used measurements of trichloroacetic acid in urine in national databases of workplace surveillance programs to identify exposed subjects for a cohort study of cancer risk (Tola et al. 1980; Axelson et al. 1994; Anttila et al. 1995; Hansen et al. 2001). Unfortunately, these workers were exposed to other solvents that also produce trichloroacetic acid as a metabolite, so there is an unknown amount of misclassification. In addition, urinary biomarkers were measured in only a fraction of the subjects studied. EPA (2001b) used estimates of exposure to trichloroacetic acid to calculate trichloroethylene exposures based on the reported relationship between trichloroethylene in air and trichloroacetic acid in urine. Unfortunately, the validity of the use of these data rests on some very weak assumptions. The first is that there is a strong relationship between trichloroethylene in air and trichloroacetic acid in urine, for which there is no strong supporting evidence (summarized by Axelson et al. 1978; also see Chapter 3).

Second, the estimates of trichloroacetic acid were not from the entire study time period, and the duration of exposure of study subjects was not known. In addition, assuming that all the exposures were purely trichloroethylene, the estimated trichloroethylene exposures were low (1-10 parts per million for most subjects).

FINDINGS AND RECOMMENDATIONS

An epidemiologic study can provide useful data on risk per unit of exposure only when the exposures have been measured or the intensity can be reasonably inferred on the basis of the exposure circumstances. Studies with small exposure groups with limited or low-intensity exposures provide little useful data on the risk from exposures. The most useful studies for exposure-risk analysis have reasonable numbers of subjects with a wide range of exposures of sufficient duration. The studies by Henschler et al. (1995), Vamvakas et al. (1998), and Bruning et al. (2003) potentially identify the upper end of the exposure-risk relationship for trichloroethylene. The exposures were high and were not confounded by exposure to other chlorinated hydrocarbons. Given the physical constraints of reasonable workplace exposures, Henschler et al. (1995) represented the upper boundary of likely total exposures.

However, because of difficulties and uncertainties with making quantitative estimates of exposure, the committee does not believe there are any currently available epidemiologic data suitable for conducting exposure-response modeling for quantifying cancer risks. Crude approaches such as those used by EPA (2001b) are appropriate for checking the reasonableness of predictions from models based on animal bioassays but are not suitable as a primary means of quantifying risks.

Recommendations:

- Consideration should be given to developing a database that compiles the study designs and results of all potentially relevant epidemiologic studies. Such a database can be used as a tool to conduct formal evaluations of characteristics of studies, conduct meta-regression analyses using study characteristics and results, and develop tables for presentation. Tables that summarize the essential design and exposure characteristics of the epidemiologic studies should be included in risk-assessment documentation.
- Epidemiologic studies should be analyzed to discriminate the amount of exposure experienced by the study population and used in classification schemes for meta-analyses (see below).
- Despite the fact that no single study can provide estimates of exposure-response patterns, the epidemiologic meta-analysis can make use of approximate “meta-levels” of exposure. An analysis of exposure should evaluate the quality of all exposure assignments and ensure that all relevant exposure data for each population were used. Studies that used the same base populations should be identified because they might be combined and their exposure information shared.
- The statistical power of each study should be assessed, given the likely percentage of the study population exposed and, where possible, the intensity of exposures. These findings could be plotted according to the methods of Beaumont and Breslow (1981).Because there are no studies with good exposure assessments for trichloroethylene, it is important to begin a prospective study on a suitable cohort that could provide the missing quantitative relationship

between long-term trichloroethylene exposure and disease risk. Such a study should include an initial retrospective exposure assessment and state-of-the-art prospective exposure assessment, including the latest exposure biomarkers of trichloroethylene metabolites and biomarkers of early effects. It may be necessary to study cohorts outside the United States.

The two meta-analyses reviewed by the committee have limitations. The meta-analysis by Kelsh et al. includes several studies that were not available at the time Wartenberg et al. performed their analysis. The new studies appear to have made the excess risk of kidney cancer more robust in the sense that the excess risk observed in the meta-analysis no longer depended solely on inclusion of the Henschler study. The Kelsh et al. analysis uses statistical methods that are more consistent with modern methods for meta-analysis than the Wartenberg et al. analysis. However, the meta-analysis by Kelsh appears to have excluded several studies that Wartenberg et al. included in their analysis. Both analyses inappropriately analyze case-control studies and cohort studies separately and used subjective assessments of quality to exclude or categorize studies. Thus, the committee judges that neither the Wartenberg et al. (2000) nor the Kelsh et al. (2005) analyses should be used for hazard characterization purposes in risk assessments for trichloroethylene.

Recommendation: The following guidelines should be used to perform a new meta-analysis of the cancer risks associated with trichloroethylene:

- Study identification
  - –A thorough search of the literature must be conducted to make sure that no relevant studies are overlooked. An important effort is to identify studies that could have investigated the risks associated with trichloroethylene.
  - –An analysis of publication bias should be conducted.
- Study selection
  - –As much as possible, all relevant studies should be included in the analysis. Any exclusion should be clearly explained and should be based on objective criteria (e.g., studies in which it was unclear that the study population was actually exposed [e.g., dry cleaning workers]).
- Weighting and classifying schemes
  - –Subjective quality scoring (e.g., tiers, groups) should not be used in the analysis. Instead, studies should be classified in terms of their characteristics, such as studies in which exposure to trichloroethylene was well documented or based on the study's design (e.g., cohort, case-control). These study characteristics should be examined as possible reasons for any observed heterogeneity, and meta-regression should be carried out, if deemed feasible.
- Analysis
  - –Both case-control and cohort studies should be included and combined unless this introduces substantial heterogeneity into the analysis.
  - –Tests of heterogeneity should be performed for all analyses. If heterogeneity is found to exist then a thorough search should be made to determine whether there are any explanations for the heterogeneity, such as differences in population exposures.
  - –Fixed and random effects models should be fitted to the data. If there is no evidence for heterogeneity, then fixed models may be preferred, but it is still appropriate to report results from random effects models as well.

–A sensitivity analysis should be performed in which each study is excluded from the analysis one at a time to determine whether any study significantly influences the findings. The findings from the meta-analysis should be viewed cautiously if they are highly dependent on the inclusion of one or two studies, and these studies have severe methodological limitations.

3

Kidney Toxicity and Cancer

This chapter reviews information on the effects of trichloroethylene on the kidney, with emphasis on information generated since the U.S. Environmental Protection Agency released its draft health risk assessment on this chemical (EPA 2001b). The review focuses on scientific issues raised during the review process that have relevance in carrying out a human health risk assessment. Studies published before the draft risk assessment are sometimes discussed to provide the context of current knowledge. Non-cancer and cancer toxicity are addressed separately; toxic effects of trichloroethylene on the nephron tubule have been proposed to have a role in cancer development, functioning as a promoter. That role is considered later in this chapter.

ROLE OF METABOLISM IN RENAL EFFECTS

Trichloroethylene nephrotoxicity, like that of several haloalkenes, is associated with a multistep metabolic pathway that includes hepatic or renal glutathione *S*-conjugate formation, enzymatic hydrolysis of the glutathione *S*-conjugates to cysteine *S*-conjugates, and renal uptake of cysteine *S*-conjugates. It is generally accepted that the cysteine *S*-conjugate *S*-(1,2-dichlorovinyl)-L-cysteine is the penultimate nephrotoxicant. *S*-(1,2-Dichlorovinyl)-L-cysteine can undergo bioactivation by renal cysteine *S*-conjugate β-lyase to reactive species (Figure 3-1), whose reaction with cellular proteins is associated with cell damage and death (Dekant et al. 1987, 1991; Pähler et al. 1999). A second pathway of haloalkene *S*-conjugates' bioactivation and toxification involving sulfoxidation of haloalkene cysteine and mercapturic acid conjugates has been identified (Sausen and Elfarra 1991; Park et al. 1992; Lash et al. 1994; Werner et al. 1995a,b, 1996; Birner et al. 1998). Sulfoxidation of haloalkyl cysteine *S*-conjugates can constitute a toxification independent of β-lyase-mediated bioactivation (Lash et al. 1994; Werner et al. 1995a,b, 1996; Birner et al. 1998). Lash et al. (2000a,b) extensively reviewed biotransformation and bioactivation of trichloroethylene. Since then, there have been additional investigations of the renal metabolism and effects of trichloroethylene, some with a focus on sulfoxidation, as well as the sulfoxidation and toxicity of other haloalkyl nephrotoxicants (see below).

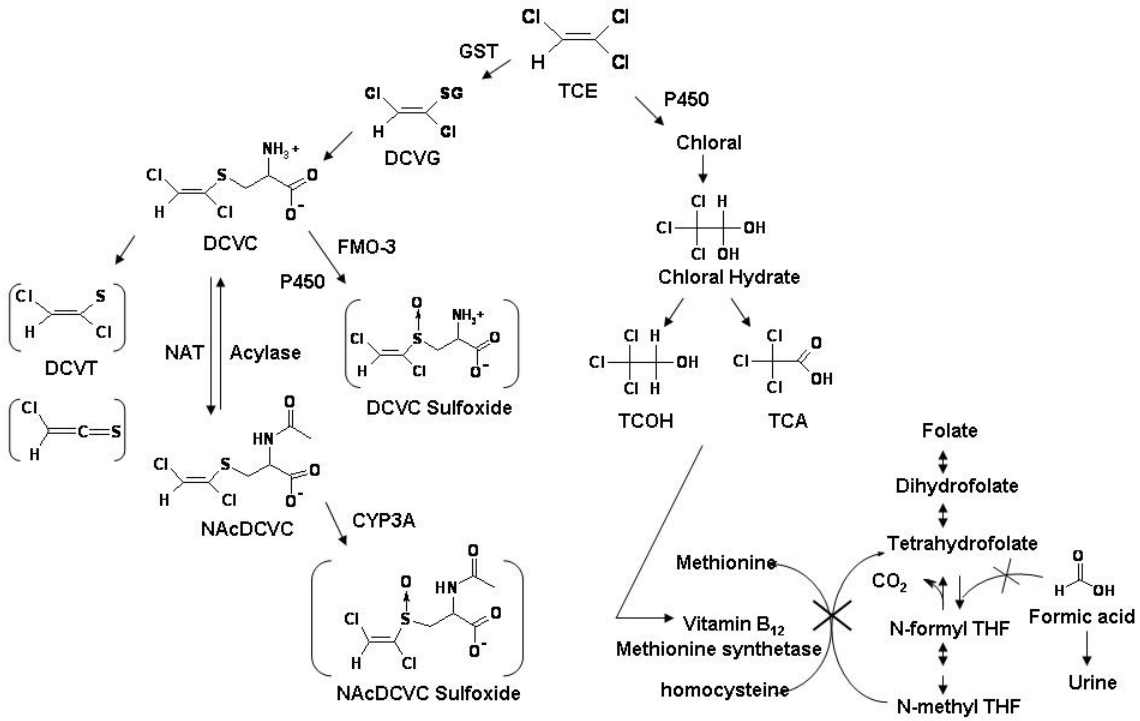

FIGURE 3-1 Composite Figure of Metabolic Pathways Relevant to Renal Toxicity Demonstrated in Mammalian Tissue (see text for references). Abbreviations: DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVT, 1,2-dichlorovinylthiol; GST, glutathions *S*-transferase; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine; NAT, *N*-acetyl transferase; TCA, trichloroacetic acid; TCOH, trichloroethanol; THF, tetrahydrofolate.

The sulfoxidation and toxicity of trichloroethylene *S*-conjugates (involving hepatic or kidney microsomal sulfoxidation of cysteine and mercapturic acid conjugates) have been clearly established (Sausen and Elfarra 1991; Lash et al. 1994; Werner et al. 1996; Krause et al. 2003; Lash et al. 2003). The first report of enzymatic trichloroethylene *S*-conjugate sulfoxidation was by Ripp et al. (1997), who demonstrated rabbit liver microsomal sulfoxidation of *S*-(1,2-dichlorovinyl)-L-cysteine. Sulfoxidation was catalyzed mainly by flavin monooxygenase, rather than by cytochrome P-450 (CYP450), and was specific for rabbit flavin monooxygenase-3 (Ripp et al. 1997). *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxidation was also catalyzed by human flavin monooxygenase-3 but not by other isoforms of flavin monooxygenase (Krause et al. 2003). Human liver microsomes also catalyzed *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation (Krause et al. 2003). Sulfoxidation was not detected with human kidney microsomes, although only one kidney sample was evaluated (Krause et al. 2003). The lack of metabolism was attributed to the low and variable concentrations of flavin monooxygenase-3 expression in kidney, which ranged from trace amounts to 1.3 pg/mg protein, compared with liver (Krause et al. 2003). *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxide, whether formed in the liver and translocated to the kidney or potentially formed renally in situ, was considered to play a possible role in trichloroethylene

nephrotoxicity (Krause et al. 2003). The mercapturic acid conjugates of dichlorovinyl cysteine, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine, also undergo sulfoxidation, as shown for rat liver microsomes (Werner et al. 1996). Unlike *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation, *S*-(1,2-dichlorovinyl)-L-cysteine mercapturate sulfoxidation was catalyzed mainly if not exclusively by CYP450, and a role for flavin monooxygenase was excluded. Specifically, rat liver microsomal *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation was catalyzed predominantly by CYP3A isoforms (Werner et al. 1996).

Haloalkyl *S*-conjugates undergo sulfoxidation primarily in the liver. *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxide was quantified after *S*-(1,2-dichlorovinyl)-L-cysteine incubation with microsomes from human liver but was not detected in microsomes from human kidney (Krause et al. 2003). Sulfoxidation of both *S*- and *N*-acetyl cysteine conjugates of *cis*- and *trans*-1,3-dichloropropene was detected in pig liver but not in rat kidney microsomes (Park et al. 1992). Nevertheless, mercapturate sulfoxidation by human kidney microsomes has been observed, albeit at rates much slower than for liver microsomes (Altuntas et al. 2004). Whether microsomes from human liver or kidney catalyze the sulfoxidation of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine, and the relative activities, remains unknown.

In general, *S*-conjugate sulfoxidation might be mediated by CYP or by flavin monooxygenase. For example, sulfoxidation of *S*-allyl-L-cysteine and *S*-benzyl-L-cysteine and, at a lower rate, *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2,2-trichlorovinyl)-L-cysteine, was catalyzed by flavin monooxygenases (Ripp et al. 1997; Krause et al. 2003). In contrast, sulfoxidation of *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine, *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine, *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine, *N*-acetyl-*S*-(1,1-difluoro-2-fluoromethoxy-2-[trifluoromethyl]ethyl)-L-cysteine, and *N*-acetyl-*S*-(1-fluoro-2-fluoromethoxy-2-[trifluoromethyl]vinyl)-L-cysteine was catalyzed mainly by CYP450 (Werner et al. 1995a,b, 1996; Altuntas et al. 2004). The relative contribution of CYP450 and flavin monooxygenase toward cysteine *S*-conjugate *S*-oxidation depends on the conjugate structure. Generally, nucleophilic sulfur atoms are oxidized preferentially by flavin monooxygenase, whereas nonnucleophilic sulfur atoms are oxidized preferentially by CYP450 (Ripp et al. 1997; Damani and Houdi 1988). Thus, cysteine conjugates with more nucleophilic sulfur atoms (*S*-allyl-L-cysteine, *S*-benzyl-L-cysteine) were much better microsomal flavin monooxygenase substrates in human kidney and liver and in rabbit liver than were those with less nucleophilic sulfur atoms (the various haloalkyl cysteine and mercapturic acid conjugates) (Ripp et al. 1997; Krause et al. 2003). This is likely because the sulfur atoms of allyl and benzyl compounds are more nucleophilic than that of vinyl compounds and because flavin monooxygenases tend to oxidize strong nucleophiles (Damani and Houdi 1988). Lipophilicity might also affect haloalkene *S*-conjugate sulfoxidation by flavin monooxygenase. *S*-Benzyl-L-cysteine is relatively lipophilic, with a nucleophilic sulfur atom, and has been shown to be a selective substrate for flavin monooxygenase (Sausen et al. 1993). *N*-Acetyl-*S*-(1-fluoro-2-fluoromethoxy-2-[trifluoromethyl]vinyl)-L-cysteine has a vinylic sulfur atom as well as strong electron-withdrawing fluorine atoms, which make the sulfur atom much less nucleophilic than those of *S*-allyl-L-cysteine, *S*-benzyl-L-cysteine, *S*-(1,2-dichlorovinyl)-L-cysteine, and *S*-(1,2,2-trichlorovinyl)-L-cysteine. *N*-Acetyl-*S*-(1,1-difluoro-2-fluoromethoxy-2-[trifluoromethyl]ethyl)-L-cysteine and *N*-acetyl-*S*-(1-fluoro-2-fluoromethoxy-2-[trifluoromethyl]vinyl)-L-cysteine are less lipophilic than *S*-benzyl-L-cysteine, rendering them

theoretically less susceptible to flavin monooxygenase sulfoxidation, potentially also explaining the lack of flavin monooxygenase activity toward their sulfoxidation.

Rat liver microsomal *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-2,2-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation was catalyzed predominantly, if not exclusively, by CYP3A isoforms (Werner et al. 1996). This conclusion was based on induction of sulfoxidation by phenobarbital and dexamethasone, inhibition by troleandomycin, and correlation with CYP3A activity. Indeed, CYP3A has been shown to be the predominant CYP isoform catalyzing the rat or human liver microsomal sulfoxidation of all haloalkyl mercapturic acid conjugates studied to date, including *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl)-L-cysteine, *N*-acetyl-2,2-*S*-(1,2-dichlorovinyl)-L-cysteine, *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine, *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine, *N*-acetyl-*S*-(1,1-difluoro-2-fluoromethoxy-2-[trifluoromethyl]ethyl)-L-cysteine, and *N*-acetyl-*S*-(1-fluoro-2-fluoromethoxy-2-[trifluoromethyl]vinyl)-L-cysteine (Werner et al. 1995a,b, 1996; Altuntas et al. 2004), which has been confirmed with cDNA-expressed CYP450s (Werner et al. 1995b; Altuntas et al. 2004). The role of CYP3A in sulfoxidation, together with the polymorphic expression of CYP3A5 in humans, raises the possibility of pharmacogenetic differences in sulfoxidation and hence toxicity in persons exposed to trichloroethylene. Indeed, sulfoxidation of *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine, *N*-acetyl-*S*-(1,1-difluoro-2-fluoromethoxy-2-[trifluoromethyl]ethyl)-L-cysteine, and *N*-acetyl-*S*-(1-fluoro-2-fluoromethoxy-2-[trifluoromethyl]vinyl)-L-cysteine was also catalyzed by expressed CYP3A5 as well as by CYP3A4 (Werner et al. 1995b; Altuntas et al. 2004). These studies showing CYP3A-catalyzed mercapturate sulfoxidation were performed in vitro. The first evidence for the role of CYP3A in any *S*-conjugate sulfoxidation in rats in vivo was recently obtained with a related haloalkene (Sheffels et al. 2004).

Sulfoxidation of trichloroethylene *S*-conjugates can constitute a toxification pathway independent of β-lyase-mediated bioactivation (Sausen and Elfarra 1991; Lash et al. 1994; Werner et al. 1995a,b, 1996; Birner et al. 1998). Sulfoxides of trichloroethylene *S*-conjugates are stable but can react readily with nonprotein thiols. Thus, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide and *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide react spontaneously with glutathione as an electrophile and Michael acceptor (Sausen and Elfarra 1991; Ripp et al. 1997; Rosner and Dekant 1999). *N*-Acetyl-1,2-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide reactivity is greater than that of other mercapturate sulfoxides, including those of *N*-acetyl-2,2-*S*-(1,2-dichlorovinyl)-L-cysteine, *S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide, and *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine, which react only slowly or require bioactivation by glutathione *S*-transferase for conjugate formation (Ripp et al. 1997; Rosner et al. 1998; Rosner and Dekant 1999).

Toxicity of the *S*-conjugate sulfoxides of trichloroethylene, and other haloalkenes, has been evaluated in vitro and in vivo. Qualitatively, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide replicated the rat renal tubular cell injury also caused by *S*-(1,2-dichlorovinyl)-L-cysteine (Lash et al. 1994). Quantitatively, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide was significantly more nephrotoxic than *S*-(1,2-dichlorovinyl)-L-cysteine to isolated rat distal, but not proximal, tubular cells in vitro (Lash et al. 1994). Like *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide, the sulfoxide of the mercapturate *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl)-L-cysteine was significantly more cytotoxic than equivalent concentrations of 1,2-*S*-(1,2-dichlorovinyl)-L-cysteine in rat renal proximal tubular cells (Werner et al. 1996). Greater cytotoxicity in rat renal tubular cells of *N*-acetyl-2,2-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide, *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine

sulfoxide, and *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine compared with their corresponding mercapturic acids was also observed (Birner et al. 1995; Werner et al. 1996). In rats in vivo, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide caused the same type of renal proximal tubular cell histologic changes as trichloroethylene and *S*-(1,2-dichlorovinyl)-L-cysteine (Lash et al. 1994). *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxide, however, was significantly more nephrotoxic than *S*-(1,2-dichlorovinyl)-L-cysteine at equivalent doses (Lash et al. 1994). A recent investigation evaluated the effects of *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide on human renal proximal tubular cells (Lash et al. 2003). *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxide caused obvious morphologic abnormalities and cellular necrosis at concentrations as low as 10 μM. *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxide also caused apoptosis. Apoptosis occurred rapidly and at low toxic concentrations, whereas necrosis occurred at later incubation times and at higher sulfoxide concentrations. Compared with *S*-(1,2-dichlorovinyl)-L-cysteine, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide caused greater and more rapid depletion of both ATP and cellular glutathione than *S*-(1,2-dichlorovinyl)-L-cysteine. Less apoptosis was observed with *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide than with *S*-(1,2-dichlorovinyl)-L-cysteine, which was attributed to the more rapid depletion of ATP. These results suggested a role for both *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide in human renal tubular cell toxicity.

Other haloalkyl mercapturate sulfoxides demonstrate similar characteristics. *N*-Acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine sulfoxide was significantly nephrotoxic in rats in vivo (Birner et al. 1998). More recently, the effects of the cysteine-*S*-, mercapturic acid, and corresponding sulfoxide conjugates of the nephrotoxicant fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether were compared in human proximal tubular cells (Altuntas et al. 2003). Both *S*-(1,1-difluoro-2-fluoromethoxy-2-[trifluoromethyl]ethyl)-L-cysteine sulfoxide and (*Z*)-*N*-acetyl-*S*-(1-fluoro-2-fluoromethoxy-2-[trifluoromethyl]vinyl)-L-cysteine sulfoxide caused greater cytotoxicity than the corresponding equimolar cysteine conjugates.

Toxicity of trichloroethylene *S*-conjugate sulfoxides occurs via a mechanism independent of β-lyase. Whereas the β-lyase inhibitor aminooxyacetic acid partially protected against *S*-(1,2-dichlorovinyl)-L-cysteine renal toxicity in vitro and in vivo, it failed to protect against *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide toxicity in both settings (Lash et al. 1994). Similarly, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine sulfoxide toxicities also were not blocked by aminooxyacetic acid (Werner et al. 1996). The α-methyl analog of *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine sulfoxide, which is not a substrate for renal β-lyase, also caused renal tubular necrosis in rats in vivo (Birner et al. 1998). *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxidation has been proposed as a mechanism to explain the observation that the D- and L- isomers of *S*-(1,2-dichlorovinyl)-L-cysteine are nearly equally nephrotoxic in rats, yet only the L-isomer is a substrate for β-lyase (Sausen and Elfarra 1991). Thus, both β-lyase-dependent metabolism of cysteine *S*-conjugates, and CYP450- or flavin monooxygenase-dependent sulfoxidation of cysteine *S*-conjugates or their mercapturates, can contribute to the bioactivation and renal toxicity of trichloroethylene and other haloalkenes.

Several questions remain unaddressed, the answers to which might have important implications for human trichloroethylene biotransformation, toxification, and individual susceptibility. Sulfoxides are more potent nephrotoxicants than their parent *S*-conjugates. Whereas rat liver microsomes catalyze *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation, and human liver microsomes catalyze *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation, whether human liver microsomes form *N*-acetyl-*S*-(1,2-

dichlorovinyl)-L-cysteine sulfoxides remains unknown. The enzymes responsible for human liver (and kidney, if extant) *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation, and particularly the role of CYP3A4 and CYP3A5, remain unknown. Interindividual variability in human *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation remains unknown. CYP3A5 is polymorphic for high expression in Caucasian (30%); Japanese (30%); Chinese (40%); and African American, Southeast Asian, Pacific Islander, and Southwestern American Indian (50%) populations (Hustert et al. 2001; Kuehl et al. 2001; see OMIM 2006a). Assuming that, like rat CYP3A, human CYP3A catalyzes these reactions, together with human CYP3A5 polymorphic expression, suggests that the potential exists for pharmacogenetic differences in sulfoxidation and hence susceptibility to toxicity. This remains unknown, as does the ability of human kidney (which constitutively expresses CYP3A as the major CYP isoform) to catalyze *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation.

More fundamentally, the existence of *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxidation in vivo has not been documented either in rats or in humans. Myriad investigations of in vivo trichloroethylene disposition in rodents and humans after controlled as well as occupational exposure (Birner et al. 1993; Bernauer et al. 1996; Bruning et al. 1998; Bloemen et al. 2001) were evaluated, including one with 10 metabolites of *S*-(1,2-dichlorovinyl)-L-cysteine (Bloemen et al. 2001); none evaluated the potential existence of trichloroethylene *S*-conjugates sulfoxides in urine. Similarly, little is known about sulfoxidation in vivo for any nephrotoxic haloalkene. Only two reports have evaluated sulfoxidation in vivo. In rats, *N*-acetyl-*S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine sulfoxide was qualitatively identified in urine after administration of hexachlorobutadiene but was not quantified (Birner et al. 1995). *N*-Acetyl-*S*-(1,1-difluoro-2-fluoromethoxy-2-[trifluoromethyl]ethyl)-L-cysteine sulfoxide was identified and quantified in the urine of rats given fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (Sheffels et al. 2004). Although sulfoxidation was apparently a quantitatively small fraction of the overall metabolism, it appeared to be a toxicologically significant route of biotransformation of fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether and its *S*-conjugates. *S*-(1,2-Dichlorovinyl)-L-cysteine sulfoxide and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide were shown to be formed by rodent liver microsomes (Werner et al. 1996; Ripp et al. 1997) and to be highly reactive renal tubular cell nephrotoxicants in rats in vitro and in vivo (Sausen and Elfarra 1991; Lash et al. 1994, 2003 Rosner and Dekant 1999) and were proposed as important determinants of trichloroethylene and *S*-(1,2-dichlorovinyl)-L-cysteine nephrotoxicity (Krause et al. 2003; Lash et al. 2003); yet no published studies have evaluated *S*-conjugate sulfoxidation from trichloroethylene or *S*-(1,2-dichlorovinyl)-L-cysteine, either in rats or in humans, or the toxicologic significance of the conjugates. Such studies may be complicated, however, by the reactivity of *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide and *N*-acetyl-1,2-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (Sausen and Elfarra 1991; Ripp et al. 1997; Rosner and Dekant 1999), which might therefore not be excreted unchanged. For example, when rats were administered *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide, the glutathione conjugate of this sulfoxide was excreted in bile (Sausen and Elfarra 1991; Rosner and Dekant 1999). Therefore, measurement of the glutathione conjugate of *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide or its metabolites may provide a method to assess *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide formation after trichloroethylene or *S*-(1,2-dichlorovinyl)-L-cysteine exposure in vivo.

NON-CANCER TOXICITY

Animal Studies

Tubular Toxicity

Trichloroethylene has been shown to cause toxicity to renal tubules in bioassay studies, and mechanisms of this toxicity have been pursued in experimental studies. Lash et al. (2000b) reviewed mechanistic studies and those will not be recapitulated here. The committee directed its efforts to studies since that review.

Trichloroethylene and *S*-(1,2-dichlorovinyl)-L-cysteine are toxic to primary cultures of rat proximal and distal tubular cells (Cummings et al. 2000). Glutathione-related enzyme activities were well maintained in the cells, whereas CYP activities were not. The response to *S*-(1,2-dichlorovinyl)-L-cysteine was greater than the response to trichloroethylene; however, the proximal and distal tubule cells had similar responses even though the proximal tubule is the target in vivo. The authors attributed this to the fact that the proximal tubule is exposed before the distal tubule in vivo and to possible differences in uptake transporters. They did not address the extent to which transporters were maintained in the cultured cells.

The same group also assessed the toxicity of trichloroethylene and its metabolites *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)glutathione using in vitro techniques (Lash et al. 2001b). Their goal was to determine whether in vitro techniques are valid indicators of species-, sex-, and tissue-related differences in sensitivity. Experiments using isolated cells were performed only with tissues from Fischer 344 rats, and lactate dehydrogenase release was used as the measure of cellular toxicity. The effects were greater in males. *S*-(1,2-Dichlorovinyl)-L-cysteine and trichloroethylene had similar effects, but *S*-(1,2-dichlorovinyl)glutathione exhibited increased efficacy compared with trichloroethylene and *S*-(1,2-dichlorovinyl)-L-cysteine. Mitochondrial toxicity was assessed in both Fischer 344 rats and $B6C3F_1$ mice. Renal mitochondria from male rats and mice responded similarly; a greater effect was seen in female mice. Thus, although these studies show *S*-(1,2-dichlorovinyl)-L-cysteine to be more toxic than trichloroethylene and *S*-(1,2-dichlorovinyl)glutathione, the magnitude of the effect was not much different and species differences are not consistent with the effects observed in long-term bioassays. This suggests that in vitro data be used with caution in risk assessment, being mindful that in vitro experiments avoid in vivo pharmacokinetic and metabolic processes.

In LLC-PK1 cells, *S*-(1,2-dichlorovinyl)-L-cysteine causes loss of mitochondrial membrane potential, mitochondrial swelling, release of cytochrome *c*, caspase activation, and apoptosis (Chen et al. 2001). Thus, *S*-(1,2-dichlorovinyl)-L-cysteine is toxic to mitochondria, resulting in either apoptosis or necrosis. *S*-(1,2-Dichlorovinyl)-L-cysteine-induced apoptosis also has been reported in primary cultures of human proximal tubule cells (Lash et al. 2001a).

Korrapati et al. (2005) builds upon a series of investigations of hetero- (by $HgCl_2$) and homo-(by *S*-(1,2-dichlorovinyl)-L-cysteine, 15 mg/kg) protection against a lethal dose of *S*-(1,2-dichlorovinyl)-L-cysteine (75 mg/kg), in which priming, or preconditioning, was said to augment and sustain cell division and tissue repair, hence protecting against the subsequent lethal *S*-(1,2-dichlorovinyl)-L-cysteine dose (Vaidya et al. 2003a,b,c). Korrapati et al. (2005) showed that a lethal dose of *S*-(1,2-dichlorovinyl)-L-cysteine downregulates phosphorylation of endogenous retinoblastoma protein (pRb), which is considered critical in renal proximal tubular and mesangial cells for the passage of cells from G_1 to S-phase, thereby leading to a block of renal

tubule repair. Priming, in contrast, upregulated P-pRB which was sustained even after the administration of a lethal dose of *S*-(1,2-dichlorovinyl)-L-cysteine, thereby stimulating S-phase DNA synthesis, which was concluded to result in tissue repair and recovery from acute renal failure and death. While these studies are indeed fascinating, they inform more on the mechanism of autoprotection rather than on the mechanism of initial injury caused by *S*-(1,2-dichlorovinyl)-L-cysteine. In addition, the priming injury (not innocuous, as it caused 25-50% necrosis and elevated blood urea nitrogen) may have influenced the toxicokinetics of the second *S*-(1,2-dichlorovinyl)-L-cysteine injection. This remains unknown.

Mensing et al. (2002) reported on the nephrotoxicity of trichloroethylene in male Long Evans rats after 6 months of inhalation exposure (500 ppm). Results were expressed relative to urine creatinine to account for individual differences in urine volume that can affect the concentration of urine constituents. Urinary excretion of albumin was not affected (although the high end of the range was about twice that of the control group) and high-molecular-weight proteins showed an upward trend but were not significantly increased (creatinine at 36 mg/g [4-81 mg/g] versus 41 mg/g [not detected, 215 mg/g]). Increased excretion of low-molecular-weight proteins and *N*-acetylglucosaminidase was noted. The increase in *N*-acetylglucosaminidase was small (8.4 units [U]/g [5.7-8.9 U/g] versus 9.7 U/g [not detected, 12.4 U/g); the increase in low-molecular-weight proteins was 332 U/g (176-659 U/g) versus 637 U/g (293-1,910 U/g). The histopathology description does not mention tubular damage, whereas interstitial infections and glomerulonephritis are described for the treated group.

Proteinuria has long been recognized as a sign of kidney damage, and it is a reliable predictor of ultimate outcome; more recently, it has been recognized that an elevated filtered load of protein is damaging to tubules (Verhave et al. 2004; Zandi-Nejad et al. 2004). Proteinuria can be characterized as glomerular, tubular, or mixed, based on the causal defect. Proteins less than about 40 kDa or 30 Å are readily filtered at the glomerulus, and are reabsorbed in the proximal tubule. Proteins larger than 100 kDa or 55 Å are not filtered. Albumin is considered an intermediate-sized protein that is normally filtered sparingly, largely because of its negative charge being repulsed by a fixed negative charge in the glomerular barrier. The glomerular pattern is excretion of high-molecular-weight proteins, such as IgG, and indicates increased permeability or decreased selectivity of the glomerular barrier. Damage to the proximal tubule impairs reabsorption of low-molecular-weight proteins; thus, a tubular pattern is one that has increased excretion of albumin and low-molecular-weight proteins, such as α_1-microglobulin. *N*-Acetylglucosaminidase is a lysosomal protein released by tubules during processing of filtered protein. Increased amounts of *N*-acetylglucosaminidase are expected when the tubules are presented with elevated amounts of protein, and thus it is an indicator of protein load. Elevated urinary *N*-acetylglucosaminidase is not an index of cell death, as is release of alkaline phosphatase from cultured cells or release of transaminase enzymes from liver cells into the plasma. However, elevation of *N*-acetylglucosaminidase in urine is a sign of proteinuria, which is a sign of kidney malfunction (Zandi-Nejad et al. 2004).

While Mensing et al. (2002) did not report tubular toxicity, the urinary protein profile is consistent with impairment of tubule reabsorption of filtered protein and perhaps increased glomerular permeability to proteins.

Role of Formic Acid in Trichloroethylene Nephrotoxicity

Some investigators (Green et al. 1998, 2003; Dow and Green 2000) have proposed that the mode of trichloroethylene nephrotoxicity is related to formic acid. They demonstrated that exposure to either trichloroethanol or trichloroacetic acid causes increased formation and urinary excretion of formic acid (Green et al. 1998). The formic acid does not come from trichloroethylene (Figure 3-1). Rather, trichloroethylene (or a metabolite) causes a functional depletion of vitamin B_{12}, which is required for the methionine salvage pathway of folate metabolism. Vitamin B_{12} depletion results in folate depletion. Folate is a cofactor in one-carbon metabolism and depletion of folate allows formic acid to accumulate, and then to be excreted in the urine (Dow and Green 2000).

The effects of trichloroethanol-induced formic acid accumulation were determined in a 1-year chronic toxicity study in male Fischer 344 rats (Green et al. 2003). Trichloroethanol was administered in drinking water to achieve a urine formic acid concentration similar to that found in rats exposed by inhalation to trichloroethylene at 500 parts per million (ppm). The pathology of formic acid (induced by trichloroethanol administration) is initially increased tubular basophilia and hyaline drop accumulation (12-16 weeks) followed by tubular degeneration at 40 weeks ("increased cellular eosinophilia, tubular vacuolation and intra-tubular cast formation") and an increased amount of pigmentation in the S2 portion of the proximal tubules and hyaline droplet accumulation. At 52 weeks, hyaline droplet and tubular degeneration were not found, but increased tubular pigmentation was observed. It was also noted that foci of "atypical" tubular hyperplasia occurred in two of the trichloroethanol-treated rats. The authors stated these changes were consistent with the nephrotoxicity seen in the 2-year cancer bioassays.

Results from the National Toxicology Program's 2-year cancer bioassays of trichloroethylene administered by gavage to rats and mice are provided in Tables 3-1 and 3-2. Nonneoplastic kidney lesions were found in all animals dosed for 2 years, including mice that did not develop kidney cancer (NTP 1988, 1990). In rats, both studies noted cytomegaly and karyomegaly of tubular cells in the area of the corticomedullary border (specified as pars recta by NTP [1990], which is situated in the corticomedullary region). Cytomegaly and karyomegaly were seen early in the bioassays and there were signs of these changes in the 13 week study (NTP 1988) (which were noted on reexamination of the slides after changes were seen in the 2-year bioassay); cytomegaly was noted at 26 weeks in (NTP 1990). Kidneys with more extensive damage had similar changes in cortical area. Both reports noted additional lesions: dilation of tubules and loss of tubular cells lining the basement membrane ("stripped appearance" [NTP 1988] or flattening of these cells [NTP 1990]). This toxic nephropathy was infrequent before 52 weeks but then increased in severity with longer exposure. Only NTP (1990) commented on intratubular material and noted that the tubules were empty or "contained wisps of eosinophilic material."

Maltoni et al. (1988) reported cancer bioassays after inhalation exposure of Sprague-Dawley rats and Swiss and B6C3F_1 mice to trichloroethylene (see Table 3-3). No renal effects were reported for mice, but renal adenocarcinomas were found in male rats at the high dose (600 ppm) at 2 years. Male rats also experienced cytokaryomegaly or megalonucleocytosis (77% of the high-dose group and 17% of the medium-dose group (300 ppm). There was no indication of pathology at earlier times.

TABLE 3-1 Summary of Renal Toxicity and Tumor Findings in Gavage Studies of Trichloroethylene by NTP (1990)

		Cytomegaly and Karyomegaly		Tumor Incidence
Sex	Dose (mg/kg)[a]	Incidence	Severity[b]	(overall; survival)
13-wk study, F344/N rats				
Male	0, 125, 250, 500, 100	Tissues not evaluated	—	None reported in
	2,000	8/9	Minimal/mild	this study
Female	0, 62.5, 125, 250, 500	Tissues not evaluated	—	
	1,000	5/10	Equivocal/minimal	
13-wk study, B6C3F$_1$ mice				
Male	0, 375, 750, 1,500	Tissues not evaluated	—	None reported in
	3,000	7/10[c]	Mild/moderate	this study
	6,000	—[d]	—	
Female	0, 375, 750, 1,500	Tissues not evaluated	—	
	3,000	9/10	Mild/moderate	
	6,000	1/10	Mild/moderate	
103-wk study, F344/N rats				
Male	0	0%	0	0/48; 0/33
	500	98%	2.8	0/49; 0/20
	1,000	98%	3.1	3/49; 3/16[e]
Female	0	0%	0	0/50
	500	100%	1.9	0/49
	1,000	100%	2.7	1/48
103-wk study, B6C3F$_1$ mice				
Male	0	0%	0	1/49
	1,000	90%	1.5	1/50
Female	0	0%	0	None
	1,000	98%	1.8	None

[a]Corn oil vehicle.
[b]Numerical scores reflect the average grade of the lesion in each group (1, slight; 2, moderate; 3, well marked; and 4, severe).
[c]Observed in four mice that died after 7-13 wk and in three that survived the study.
[d]All mice died during the first week.
[e]$P = 0.028$

The lesions due to formic acid (induced by trichloroethanol administration) and trichloroethylene exposure differ in the nature and the time course of the lesions. They are similar in that the same region of the kidney is affected. However, that region of the kidney is most often affected by nephrotoxic chemicals and by hypoxia and ischemia. Green et al. (2003) did not observe flattening or loss of tubular epithelial cells nor did they report tubular dilation. Hyaline droplets and tubular degeneration were found at 40 weeks, but not at 52 weeks, which is when tubular degeneration (albeit with different characteristics) was noted in the cancer bioassays. Toxic nephropathy was infrequent before 52 weeks, but then increased in severity with longer exposure (NTP 1990). Intratubular cast formation was noted as part of the tubular degeneration following exposure to formic acid (produced by trichloroethanol exposure), but with trichloroethylene exposure tubules were described as empty or containing "wisps" of material. Because dosing with trichloroethanol was selected to achieve the concentrations observed after exposure to daily inhalation of trichloroethylene at 500 ppm, similar to that used

TABLE 3-2 Summary of Toxicity and Tumor Findings in Gavage Studies of Trichloroethylene by NTP (1988)

Sex	Dose (mg/kg)[a]	Cytomegaly	Toxic Nephropathy	Adenoma	Adenocarcinoma
2-yr study, ACI rats					
Male	0	0/50	0/50	0/50	0/50
	500	40/49 (82%)	18/49 (37%)	0/49	1/49
	1,000	48/49 (98%)	18/49 (37%)	0/49	0/49
Female	0	0/48	0/48	0/48	0/48
	500	43/47 (91%)	21/47 (45%)	2/47	1/47
	1,000	42/43 (98%)	19/43 (44%)	0/43	1/43
2-yr study, August rats					
Male	0	0/50	0/50	0/50	0/50
	500	46/50 (92%)	10/50 (20%)	1/50	1/50
	1,000	46/49 (94%)	31/49 (63%)	1/49	0/49
Female	0	0/49	0/49	1/49	0/49
	500	46/48 (96%)	8/48 (17%)	2/48	2/48
	1,000	50/50 (100%)	29/50 (58%)	0/50	0/50
2-yr study, Marshall rats					
Male	0	0/49	0/49	0/49	0/49
	500	48/50 (96%)	18/50 (36%)	1/50	0/50
	1,000	47/47 (100%)	23/47 (49%)	0/47	1/47
Female	0	0/50	0/50	1/50	0/50
	500	46/48 (96%)	30/48 (63%)	1/48	1/48
	1,000	43/44 (98%)	30/44 (68%)	0/44	1/44
2-yr study, Osborne-Mendel rats					
Male	0	0/50	0/50	0/50	0/50
	500	48/50 (96%)	39/50 (78%)	6/50	0/50
	1,000	49/50 (98%)	35/50 (70%)	1/50	1/50
Female	0	0/50	0/50	0/50	0/50
	500	48/50 (96%)	30/50 (60%)	0/50	0/50
	1,000	49/49 (100%)	39/49 (80%)	1/49	0/49

[a]Corn oil vehicle.

in the Maltoni et al. (1988) study, it is noteworthy that the histopathologic descriptions of the Maltoni et al. study differ from those of the Green et al. (2003) study.

Dow and Green (2000) noted that trichloroacetic acid also induced formic acid accumulation in rats. If formic acid is the actual trichloroethylene nephrotoxicant, then trichloroacetic acid would be expected to cause similar pathology. Mather et al. (1990) reported an increase of kidney-weight to body-weight ratio in rats after 90 days of exposure to trichloroacetic acid in drinking water at 5,000 ppm but reported no histopathologic changes in the kidney. DeAngelo et al. (1997) reported no effects of trichloroacetic acid on kidney weight or histopathology in rats in a 2-year cancer bioassay. The amount of formic acid produced after administration of trichloroethanol or trichloroacetic acid in drinking water was similar at 2-4 weeks (about 20 mg/day for each compound) and was the same at the two doses used (1 and 5 g/L for trichloroacetic acid and 0.5 and 1.0 g/L for trichloroethanol). The studies with trichloroethanol were carried out for a longer time and excretion of formic acid at the high dose increased to about 60 mg/day. However, because the dose-response relationship was lost, folate

TABLE 3-3 Summary of Toxicity and Tumor Findings in Inhalation Studies of Trichloroethylene by Maltoni et al. (1988)

Sex	Concentration (ppm)	Megalonucleocytosis	Renal Adenocarcinoma
2-yr study, Sprague-Dawley rats			
Male	0	—	—
	100	—	—
	300	16.9%	—
	600	77.7%	3.1%
Female	0	—	—
	100	—	—
	300	—	—
	600	—	0.7%
78-wk study, Swiss mice			
Male	0	—	—
	100	—	—
	600	—	—
Female	0	—	—
	100	—	—
	600	—	—
78-wk study, B6C3F$_1$ mice			
Male	0	—	—
	100	—	—
	600	—	—
Female	0	—	—
	100	—	—
	600	—	—

was added to the regime of the low-dose animals, and this decreased their formic acid excretion. The formic acid exposure in the groups exposed to trichloroacetic acid at 5,000 ppm in the Mather et al. (1990) and DeAngelo et al. (1997) studies would be similar to that of the group treated at 1 g/L in the Green et al. (2003) study.

In summary, on the basis of dissimilarities between the pathologic responses of the kidney to formic acid and trichloroethylene and the lack of nephrotoxicity from trichloroacetic acid, which also results in formic acid production, it is difficult to accept formic acid formation as a mechanism or mode of action for trichloroethylene.

$\alpha_{2\mu}$-Globulin Accumulation

There is no evidence that trichloroethylene induces $\alpha_{2\mu}$-globulin accumulation (Goldsworthy et al. 1988), and the histopathologic effects of trichloroethylene are not consistent with that histopathology (EPA 1991). Trichloroethanol was recently reported to cause hyaline droplet accumulation and an increase in $\alpha_{2\mu}$-globulin accumulation that was insufficient to account for the hyaline droplet nephropathy (Green et al. 2003). Similar to tubular damage, the hyaline droplet accumulation was seen at 40 weeks but not at 52 weeks.

Peroxisome Proliferation

The role of peroxisome proliferation as a mode of action of trichloroethylene was considered in the review by Lash et al. (2000b). They concluded that the published literature does not support peroxisome proliferation as a mode of action for renal carcinogenesis from trichloroethylene or its metabolites.

Human Studies

Tubular Toxicity

Trichloroethylene and *S*-(1,2-dichlorovinyl)-L-cysteine have been shown to be toxic to fresh human proximal tubule cells (Cummings and Lash 2000) and *S*-(1,2-dichlorovinyl)-L-cysteine is toxic to cultures of human proximal tubule cells (Lash et al. 2001a). *S*-(1,2-Dichlorovinyl)-L-cysteine produced necrosis, apoptosis, and an increase in the percentage of cells in S phase, an indication of cell proliferation. The authors noted that effects were observed in the 10 to 100 μM range, judged to be occupationally relevant because the concentration of *S*-(1,2-dichlorovinyl)glutathione in the blood is 45 μM after 4 hours of exposure at 100 ppm.

Biological monitoring of persons who previously experienced "high" exposures to trichloroethylene (100-500 ppm) in the workplace has been performed. These studies have used generalized proteinuria and urinary excretion of specific tubular proteins as an index of tubular toxicity. Bruning et al. (1999a) reported results supporting nephrotoxicity in kidney cancer patients. They compared the highly trichloroethylene-exposed group with nonexposed renal cancer patients and with healthy, unexposed controls. Both renal cancer groups were about 2.5 years postnephrectomy. In the renal cancer groups, 95% (39 of 41) of trichloroethylene-exposed patients had elevated proteinuria, 85% had tubular proteinuria, 7% had a combined pattern, and 2% had glomerular proteinuria. In comparison, only 44% (22 of 50) of the nonexposed renal cancer patients had tubular proteinuria and 2% had a combined pattern (54% had no proteinuria). The trichloroethylene-exposed patients had elevated excretion of α_1-microglobin compared with the nonexposed renal cell cancer patients. The authors concluded that their results support an initiation-promotion model, in which the repeated toxicity, evidenced by increased incidence of proteinuria, serves as the promoter for the genotoxic[1] metabolites produced via the glutathione pathway.

Bolt et al. (2004) measured α_1-microglobulin excretion in patients from the case-control study by Bruning et al. (2003) (details provided later in this chapter). Some subjects in this study were highly exposed; of the 134 with renal cell cancer, 19 reported past exposures that led to narcotic effects and 18 of the 401 controls, experienced similar effects (odds ratio [OR] = 3.71, 95% confidence interval [CI] 1.80-7.54). The study of Bolt et al. was based on urine samples obtained from 74% of the patients and 75% of the controls. They found that α_1-microglobulin excretion increased in exposed renal cancer patients compared with nonexposed patients exposed controls. Of the exposed cancer patients, 15% had normal α_1-microglobulin excretion, whereas 52% of the nonexposed patients did. On the high end, 55% of the exposed patients had α_1-

[1]Mutagenicity refers to the ability of a chemical to induce heritable mutations, whereas genotoxicity is a broader term that includes mutational end points, cytogenetic analysis, and primary DNA damage.

microglobulin excretion greater than 11 mg/L, compared with 29% of the nonexposed cases. The results of this case-control study agree with their previous study (Bruning et al. 1999a).

Proteinuria was also observed in exposed male workers who were not cancer patients (Bruning et al. 1999b). Severe tubular proteinuria was seen in 35% of exposed workers but in none of the nonexposed workers; slight tubular proteinuria was seen in 20% of exposed workers and in 2% of nonexposed workers. α_1-Microglobulin excretion was significantly increased in the exposed group compared with controls.

Green et al. (2004) measured biomarkers of the proposed formate mode of action and exposure in a group of workers currently exposed to trichloroethylene. They found that urinary excretion of albumin, total *N*-acetylglucosaminidase and formate were increased in the exposed group compared with the unexposed group. As discussed above under Animal Studies, Tubular Toxicity, elevation of *N*-acetylglucosaminidase in urine is a sign of proteinuria, and proteinuria is both a sign and a cause of kidney malfunction (Zandi-Nejad et al. 2004). The exposed workers excreted an average of 9.7 (standard deviation [SD] = 11.6) mg of albumin per g of creatinine, significantly different from the nonexposed group value of 5.5 (SD = 4.3). For a urine sample, 10-17 mg of albumin per g of creatinine is considered to be suspected albuminuria in males (15-25 in females) (De Jong and Brenner 2004). Thus, the results presented provide evidence for kidney damage at current occupational exposure conditions. Nevertheless, Green et al. (2004) state that *N*-acetylglucosaminidase does not indicate nephropathy, or damage, but rather is an indicator of functional change in the kidney.

Green et al. (2004) performed further analyses to examine the exposure-response relationship. Trichloroethylene exposure was estimated by applying the German occupational exposure limit (maximale arbeitsplatz konzentration, MAK) standard to urine trichloroacetic acid and assuming that the linear relationship holds for exposures above 100 ppm. Neither *N*-acetylglucosaminidase nor albumin concentration correlated to trichloroacetic acid and, therefore, to estimated exposure; they concluded that increased urinary albumin or *N*-acetylglucosaminidase was not related to trichloroethylene exposure.

This conclusion is predicated on the assumption that trichloroacetic acid in urine reliably estimates trichloroethylene exposure and that the relationship of urine trichloroacetic acid to trichloroethylene is linear up to 250 ppm (their highest estimate). The published literature indicates that urinary trichloroacetic acid concentration is not a reliable predictor of trichloroethylene exposure. This results from: the variability within the data, the linearity of the relationship, and genetic variations within the populations. These are discussed in the paragraphs below.

Studies reporting urinary excretion of trichloroethylene metabolites show considerable variability between individuals. Ikeda et al. (1972) measured exposure in workshops and trichloroethylene metabolites in urine from workers. They presented results as the mean of five measurements of exposure for each workshop and the mean and standard deviation of urinary trichloroethylene metabolite measurements from workers at that workshop. They did not include goodness-of-fit characterization. The variation around the data is considerable; a urine trichloroacetic acid concentration of 100 mg/L could be obtained after an exposure ranging from 20 to 60 ppm trichloroethylene and a concentration of 200 mg/L could be obtained after an exposure of 50 to 200 ppm. The MAK values Green et al. (2004) used to estimate exposure fall within the range of results of Ikeda et al. (1972). Inoue et al. (1989) used personal diffusive samplers to measure time-weighted exposures during the shift of individual workers and compared them with various metabolites (trichloroacetic acid, trichloroethanol, and its

metabolite glucuronide). They reported a correlation coefficient (r) of 0.457 for the males and females combined; the r^2 would be 0.209, indicating that about 21% of the variation of trichloroacetic acid excretion among subjects is due to trichloroethylene exposure.

The second concern is the exposure range for which metabolite excretion is linear. Ikeda et al. (1972) noted that the relationship between trichloroethylene exposure and urinary trichloroacetic acid was nonlinear at trichloroethylene concentrations above 50 ppm, reaching a plateau at 100 ppm, thus indicating saturation of trichloroacetic acid formation. Inoue et al. (1989) did not observe saturation of metabolism, and suggested it was because most of the workers were exposed to concentrations below 50 ppm. The slope of the exposure-response relationship was much flatter (0.31 for males) than that of Ikeda et al. (2.74). Fisher et al. (1998) exposed human volunteers to trichloroethylene in support of developing a physiologically based pharmacokinetic model for trichloroethylene. They reported cumulative trichloroacetic acid excretion in urine over time, not concentrations in urine. They used two exposures, 50 and 100 ppm, and two each of the males and females were exposed to both concentrations (additional subjects were exposed to only one concentration). One male and one female did not have a higher cumulative urinary excretion of trichloroacetic acid at 100 ppm. These results are consistent with a saturation of metabolism above 50 ppm for some subjects.

Genetic differences in study populations might contribute to the differences observed. The Ikeda study (1972) appears to involve Japanese workers. The subjects of the Inoue et al. (1989) paper are from China. Green et al. (2004) did not state the nationality of the study subjects, but several of the authors are located in Singapore and China, and genetic polymorphisms are known to occur for CYP2E1 within Asian populations (Hayashi et al. 1991;OMIM 2006b). Inoue et al. (1986) reported that Japanese men have higher rates of a CYP2E1-mediated reaction (toluene metabolism to hippuric acid) than Chinese men and Japanese women; they suggested the higher rates may be related to higher alcohol consumption by Japanese males. CYP3A5 is polymorphic for high expression in Caucasian (30%); Japanese (30%); Chinese (40%); and African American, Southeast Asian, Pacific Islander, and Southwestern American Indian (50%) populations (Hustert et al. 2001; Kuehl et al. 2001; OMIM 2006a).

Green et al. (2004) concluded that increased protein excretion was not related to the extent of trichloroethylene exposure as assessed by urinary trichloroacetic acid concentration. Because the relationship of urinary trichloroacetic acid concentration to ambient trichloroethylene concentration is highly variable and nonlinear, the committee does not consider urinary concentrations of trichloroacetic acid to be sufficiently reliable to use as a quantitative measure of exposure. Therefore, analyses based on urinary trichloroacetic acid measurements should not be used to conclude that trichloroethylene does not cause nephrotoxicity. Rather, weight of evidence indicates that proteinuria is occurring at current occupational exposures and that kidney damage is occurring.

Although generalized proteinuria and urinary excretion of specific tubular proteins have been used to evaluate renal tubular cell toxicity in animals and humans exposed to trichloroethylene, it should be noted that while proteinuria does result from tubular toxicity, it is not specific for trichloroethylene or tubular nephrotoxins in general (D'Amico and Bazzi 2003; Han and Bonventre 2004; Lane 2004). Proteinuria can, for example, result from nonxenobiotic tubular injury and from glomerular disease, and is also associated with diabetes, cardiovascular disease, and inflammation. Considerable effort has been directed toward identifying urinary biomarkers that detect early and subclinical acute renal tubular injury, but this remains an

unattained ideal. Development and validation of a biomarker for nephrotoxicity from trichloroethylene or, more likely, haloalkenes in general, remains an area for future investigation.

In summary, recent studies show that humans exposed to trichloroethylene have tubular proteinuria and, thus, have experienced toxic insult similar to that observed in rats.

Formate

Green et al. (2004) measured formate in urine and used it as a mode-of-action marker. They did not establish a dose-formate relationship—the study was not adequate to establish a dose-response relationship. Formate did correlate with trichloroacetic acid formation and with methylmalonic acid and glutathione *S*-transferase in urine, all of which were considered to be mode-of-action markers.

Formic acid nephrotoxicity has been reported in humans following deliberate poisonings. Hematuria is noted within a few hours, followed by acute renal failure (Rajan et al. 1985).

KIDNEY CANCER

Hazard Identification from Epidemiology Studies

The committee was charged with evaluating the strengths and limitations of the body of epidemiologic evidence on trichloroethylene and kidney cancer. The guidelines for evaluating epidemiologic studies developed in Chapter 2 are used in this assessment. To identify the relevant studies, the committee used all studies listed in previous assessments by Wartenberg et al. (2000), Kelsh et al. (2005), and the Institute of Medicine (IOM 2003). In addition, the committee reviewed materials (published and unpublished) submitted during the course of its study. Although the committee is unsure that this represents all the epidemiologic literature on kidney cancer and trichloroethylene, it suffices to illustrate the essential methodological issues.

As discussed in Chapter 2, it is important to include detailed tables and figures that summarize the main design characteristics of the epidemiologic studies in any risk assessment. Many formats can be used for that purpose. In this report, the committee used the format the Institute of Medicine (IOM 2003) developed for its Gulf War study regarding the chronic health effects from exposure to organic solvents and insecticides. Tables provide the essential design characteristics of the cohort and case-control studies and their principal findings, including all newly identified studies since publication of the IOM report.

Cohort Studies

The committee focused its evaluation on occupational cohort studies conducted in a variety of industries in which workers were exposed to trichloroethylene, including aircraft and aerospace workers (Garabrant et al. 1988; Costa et al. 1989; Blair et al. 1998; Morgan et al. 1998; Boice et al. 1999), biologically monitored workers in national programs of Scandinavia (Axelson et al. 1994; Antilla et al. 1995; Hansen et al. 2001), rubber workers (Wilcosky et al. 1984), cardboard and paperboard workers (Sinks et al. 1992; Henschler et al. 1995), uranium-

processing workers (Ritz 1999), electronics workers (Greenland et al. 1994; Chang et al. 2003), and workers in other industries. The design characteristics of 18 cohort studies are presented in Table 3-4 and selected results from the studies are provided in Table 3-5. The committee has attempted to compile a complete list of studies that provide insights into the association between exposure to trichloroethylene and kidney cancer. Estimates of relative risk for all cancer sites were not provided in all papers. For example, Shindell and Ulrich (1985) only reported on major disease categories because of the small size of the cohort.

Subjects in the studies were mostly men and their age range when they entered the cohorts was typical of working populations. Studies of dry cleaning workers were not considered because it appears unlikely that substantial numbers of them were exposed frequently to sufficient amounts of trichloroethylene (Stewart and Dosemeci 2005). In particular, trichloroethylene was not used extensively in the dry cleaning industry between the 1930s and 1960s and it was used rarely in subsequent decades, although it was used in spot stain removal throughout the century.

In the following sections, the committee identifies the critical issues to consider in evaluating cohort studies of kidney cancer.

Follow-Up and Misclassification of Incidence

Rate ratios (and power) estimated in cohort studies of kidney cancer could be underestimated if the follow-up period was not long enough to account for latency (say, mean latent periods on the order of 15-25 years). In mortality studies, further attenuations of power would occur because some incident cases could have been lost through misclassification of the cause of death. Nondifferential misclassification of the outcome in cohort studies (independent of exposure and reference groups) will lead to attenuation of the rate ratios, although the magnitude is difficult to predict. Thus, the rate ratios estimated in the mortality cohort studies of kidney cancer (e.g., Shindell and Ulrich 1985; Garabrant et al. 1988; Sinks et al. 1992; Axelson et al. 1994; Greenland et al. 1994; Blair et al. 1998; Morgan et al. 1998; Boice et al. 1999; Ritz 1999) are likely underestimated to some extent. The magnitude of this bias can be calculated theoretically if the sensitivity and specificity of attributing the cause of death as kidney cancer are known or estimated. In addition, methods for correcting the estimated relative risks as well as the confidence intervals are available and might be useful if error rates are known (Brenner and Gefeller 1993).

Statistical Power

Kidney cancer is a rare disease. In the United States, the age-adjusted rate of cancer of the kidney and renal pelvis is 12.6 per 100,000 people, and this rate varies by gender and race (SEER 2005; Table 3-6). The American Cancer Society (2006) estimates that nearly 39,000 patients will be diagnosed with kidney cancer in 2006, more than twice the number of patients expected to be diagnosed with liver cancer (see Chapter 4). Thus, only very large cohort studies would have a sufficient number of cases to provide adequate statistical power to estimate excess risks and exposure-response relationships. In particular, negative findings from cohort studies

TABLE 3-4 Selected Cohort Studies That Present Associations Between Cancer and Exposure to Trichloroethylene

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Aircraft and Aerospace Workers					
Garabrant et al. 1988	Mortality experience (1958-1982) of aircraft manufacturing workers (at least 1 day) at an aircraft manufacturing facility in San Diego County, California (with at least 4 yr of cumulative company employment).	14,067 total 11,898 men 2,169 women	U.S. general population	Employment determined through company work records and interviews; about 37% of jobs had exposure to TCE, based on 70 subjects.	SMR Age, sex, race, calendar year, duration of employment, year of death
Costa et al. 1989	Mortality experience (1955-1981) of workers in Turin, Italy, involved in manufacturing aircraft and aerospace components. Subjects were those working at the plant in 1954 and newly employed until 1981.	8,626 total 7,676 men 950 women	Italian general population	Employment determined through company work records. No exposure assessment carried out. Hazardous exposures included cutting fluids, rubber plastic paint dyes, organic solvents, paints, welding fumes, epoxy resins and hardeners, asbestos, manmade mineral fibers, and ionizing radiation (testing of components). No specific mention of TCE.	SMR Age, sex, race, calendar year

TABLE 3-4 *Continued*

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Spirtas et al. 1991; Blair et al. 1998	Incidence and mortality experience (1952-1990) of aircraft maintenance workers (at least 1 yr in 1952-1956) at Hill Air Force Base in Utah.	14,457 total 10,730 men 3,727 women	Utah white population	Industrial hygienist assessment from interviews, surveys, hygiene files, position descriptions Exposure to TCE from dipping large parts and cleaning small electrical components with squeeze bottles. Exposure scored by department or job; rank-ordered index of intensity. Other exposures: Stoddard solvent, isopropyl alcohol, 1,1,1-trichloroethane, acetone, toluene, methyl ethyl ketone, methylene chloride. Degreasing: 1950-1960, TCE replaced Stoddard solvent and carbon tetrachloride. Early 1960, concentration of TCE about 400 ppm during the usual 15 min of degreasing. This was reduced to about 200 ppm in late 1960s. After 1978: TCE replaced by 1,1,1-trichloroethane 1968: cold state solvent: TCE replaced by 1,1,1-trichloroethane.	SMR, RR (Poisson) Age, sex, calendar period
Morgan et al. 1998	Mortality experience (1950-1993) of aerospace workers (at least 6 months) at Hughes Aircraft plant in Arizona.	20,508 total (4,733 exposed) 13,742 men 6,766 women	U.S. general population	Exposure matrices generated by employees and industrial hygienists. TCE used in vapor degreasing (>50 ppm) 1952-1977 Coded as none (0), low (1), medium (4), high (9).	SMR, Cox proportional hazards model Age, sex

TABLE 3-4 *Continued*

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Boice et al. 1999	Mortality experience (1960-1996) of aircraft manufacturing workers (at least 1 yr) Lockheed Martin facility in California.	77,965 total 62,477 men 15,488 women	California general population of white workers	Abstracted from walk-through surveys, hygiene files, job descriptions. TCE used until 1966 and perchloroethylene after 1996. 70% of workers using TCE or perchloroethylene also exposed to chromates. Jobs with exposure to TCE or perchloroethylene: process equipment operator, electroplater, metal bond assembler, heat transfer, sheet metal forming. Other exposures: chromate, Perc, mixed solvents.	SMR, RR (Poisson) Age, sex, race, dates of first and last employment
Zhao et al. 2005	Mortality and cancer incidence experience of 6,107 male workers at the Santa Susana Field Laboratory (Boeing) in California. Workers were employed between 1950 and 1992 with follow-up until the end of 2001.	6,107 men	Internal comparison	Job-exposure matrix developed through assessment of the workplace. Exposure scores for TCE, benzene, polycyclic aromatic hydrocarbons, mineral oil, and hydrazine. Exposure score = intensity of exposure (none, low, medium, high) × number of years exposed summed over all jobs.	Cox model adjusted for pay type, time since hire, and age. Additional adjustments for other exposures, including hydrazine.

TABLE 3-4 *Continued*

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Other Cohort Studies					
Axelson et al. 1978, 1994	Mortality experience (1955-1986) of Swedish workers occupationally exposed during the 1950s and 1960s. Cohort included workers in manufacturing plants as well as users.	1,670 total 1,421 men 249 women	Sweden general population	Biological monitoring for U-TCA	
Wilcosky et al. 1984	Cases, age 40-84 years, selected retrospectively from a cohort of active and retired male rubber workers in a plant in Akron, Ohio, in 1964-1973; an age-stratified, 20% random sample from the original cohort served as the control group.	NA	1,336 (20% of 6,678)	Linkage of worker histories to plant solvent-use records; work in process area with known solvent use equates to exposure. Other exposures: TCE, Perc, toluene, xylenes, naphthas, ethanol, acetone, phenol.	Race-specific ORs Age
Shindell and Ulrich 1985	Mortality experience of white and nonwhite men and women, >3 months employment, 1957-1983, in a manufacturing plant in Illinois that used TCE as a degreasing agent.	2,140 white men 76 nonwhite men 430 women	External comparison with U.S. general population	None	SMR Age, sex, calendar year

TABLE 3-4 *Continued*

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Seldén and Ahlborg 1991	Mortality and incidence experience among male Swedish Armed Forces personnel possibly exposed to military aircraft fuel (MC77, MC25) during 1972 to 1974. Follow-up from 1975 to 1984 (mortality) and from 1975 to 1983 (cancer incidence). Exposure assessments from 1930 to 1983. Percent of eligible cohort included in study, 2,176/2,181 = 99.8%.	2,176 men 1,865 from Air Force	Swedish general population	Estimates of probability of exposure to different types of military aircraft fuel attributed by a senior aircraft technician from subjects' personnel files. Fuels considered: MC55, MC75, MC77, MC25. If exposure to TCE, then it would be part of the complex mixture of the aircraft fuels.	SMR, SIR Age, sex, time period
Sinks et al. 1992	Mortality experience of paperboard printing workers, >1 day employment, 1957-1988. Based on a "cluster" reported by a physician.	1,765 white men 63 black men 219 white women 3 black women	External comparison with U.S. general population; internal comparison	None	SMR and SIR Age, sex, calendar year; Nested case-control study on renal cancer

TABLE 3-4 *Continued*

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Greenland et al. 1994	Mortality experience of white men in a transformer manufacturing plant. Subjects were employed before 1985, died in the period 1969-1984, as reported by the company pension plan, and had a job history, were between ages 21 and 90 at death, ended work after 1946.	512 cases of different sites of cancer. 1,202 controls with other causes of death. Controls were excluded if they died of diseases of the blood and blood-forming organs (ICD8 280-289), mental disorders (290-315), and diseases of the digestive system (580-629). 21 cases and 68 controls did not meet the eligibility criteria.	Internal comparison	Job titles rated for exposure by industrial hygienist and created a job-exposure matrix for specific solvents, including TCE (coded as unexposed/exposed).	Logistic analyses adjusting for age, year of death, and other covariates that altered the exposure estimate by >20%
Anttila et al. 1995; Tola et al. 1980	Incidence experience (1967-1992) of workers biologically monitored for occupational exposure to halogenated solvents (1965-1982) at the Finnish Institute of Occupational Health.	3,974 total 2,050 men 1,924 women	Finland general population	Biological monitoring for U-TCA and blood metabolites of Perc and trichloroethane. Other exposures and biomonitoring: 1,1,1-trichloroethane, Perc	SIR Age, sex, time period

TABLE 3-4 *Continued*

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Henschler et al. 1995	Mortality and incidence experience of cardboard manufacturing plant, Germany, >1 yr, 1956-1975, follow-up until 1992.	183 exposed men; 169 participated in study. Control cohort of 190 unexposed men, matched on age and physical job activity to exposed cohort. Unclear how the cohorts were constituted and followed. Appears to be based on a cluster of 5 cases. Case finding may not have been adequate.	Internal comparison uses Danish rates to calculate expected. External comparison with Danish and German cancer registry incidence rates. Cause of death from hospital records; cause of death for external rates based on death certificates.	TCE exposure, used for cleaning between 1956 and 1975. In 1976, other solvents used in small quantities. Walk-through I survey. High exposures in cardboard-machine area. Lower but continuous exposures in locksmith and electrical workshops.	Internal comparison using SIRS, adjusted for age. External: SIR calculations. Body weight, height, blood pressure, intake of diuretics, smoking, and alcohol assessed.
Ritz 1999	Mortality experience (1951-1989) of male uranium-processing plant workers (at least 3 yr, with first hire in 1951-1972) in Ohio.	3,814 men	(1) External comparison with U.S. general population. (2) Internal comparison among workers monitored for exposure.	Exposure matrices generated by employees and industrial hygienists for TCE, cutting fluids, kerosene. Workers classified as not exposed or as low (1), medium (2), high (3).	SMR, RR (conditional logistic regression) Age, calendar year, time since first hired, pay type, internal and external radiation dose
Hansen et al. 2001	Incidence experience (1968-1996) in Danish workers (1947-1989) occupationally exposed.	803 total 658 men 145 women	Denmark general population. Nonrandom sample; loss of subjects because they could not be linked.	Biological monitoring for U-TCA.	SIR Age, sex, calendar year, period of first employment, employment duration

TABLE 3-4 *Continued*

Reference	Description	Study Group (No. of Subjects)	Comparison Group	Exposure Assessment and Other Relevant Exposures	Analysis and Adjustment for Potential Confounders
Raaschou-Nielsen et al. 2003	Incidence experience of workers at 347 small plants in Denmark using TCE, follow-up 1968-1997.	40,049 men and women	External comparison with Danish cancer registry	Duration of employment, year of first employment positively correlated, and number of employees negatively correlated with exposure to TCE.	External SIR calculations, adjusted for sex, age, calendar year
Chang et al. 2003	Mortality experience of workers at an electronics manufacturing plant in Taiwan employed between 1978 and 1997 and followed from 1985-1997. Subjects at the plant were identified by Bureau of Labor Insurance files, United Labor Association, and labor-insurance hospitalization data.	86,868 total 16,133 men 70,735 women	Person-years calculated from 1985 onward; external comparison with Taiwan national mortality rates	Exposures to organic solvents at the plant were due to TCE and perchloroethylene. The primary index of exposure was duration of employment at the plant. Because no company records were available, duration was based on insurance records: from inception to termination of labor insurance coverage. Durations were likely underestimated as dates of commencement (n = 6508; 7.5%) and termination (n = 6.0%) of insurance coverage were incomplete.	External SMR calculations, adjusted for sex, age, calendar year

Abbreviations: ICD, International Classification of Disease; NA, not available; OR, odds ratio; Perc, tetrachloroethylene; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TCE, trichloroethylene; U-TCA, urinary trichloroacetic acid.
Source: Adapted from IOM 2003.

TABLE 3-5 Selected Results from Cohort Studies of Kidney Cancer and Occupational Exposure to Trichloroethylene

Reference	Study Population	Exposed Cases	Estimated RR (95% CI)	Approximate Statistical Power to Detect a RR = 2
Incidence				
Sinks et al. 1992	Cardboard manufacturers, USA			
	SIR	6	3.7 (1.4-8.1)	27.9
Hansen et al. 2001	Danish workers occupationally exposed to TCE (U-TCA monitoring)	3	0.9 (0.2-2.6)	44.9
	Men, ever exposed	1	2.4 (0.03-14)	13.5
	Women, ever exposed			
Blair et al. 1998	Male aircraft maintenance workers in Utah			
	Total	15	1.6 (0.5-5.1)	81.5
	No exposure	9	1.6 (0.5-5.4)	62.8
	<5 unit-yr	9	1.4 (0.4-4.7)	67.8
	5-25 unit-yr	5	1.3 (0.3-4.7)	49.4
	>25 unit-yr	2	0.4 (0.1-2.3)	58.4
	Low intermittent exposure	12	2.1 (0.6-7.5)	63.3
	Low continuous exposure	9	2.2 (0.6-8.1)	51.4
Henschler et al. 1995	Male German cardboard manufacturers, employed >1 yr	5	7.97 (2.59-8.59)	16.3
Anttila et al. 1995	Finnish workers occupationally exposed to TCE (U-TCA monitoring)			
	Entire period since first measurement	6	0.87 (0.32-1.89)	70.4
	0-9 yr	1	0.53 (0.01-2.95)	30.8
	10-19 yr	5	1.39 (0.45-3.24)	47.2
	20+ yr	0	— (0.00-2.48)	
Axelson et al. 1994	Swedish men occupationally exposed to TCE (U-TCA monitoring)	6	1.16 (0.42-2.52)	59.6

TABLE 3-5 *Continued*

Reference	Study Population	Exposed Cases	Estimated RR (95% CI)	Approximate Statistical Power to Detect a RR = 2
Raaschou-Nielsen et al. 2003	Danish workers exposed to TCE			
	Men	93		
	Women	10		
	Duration of exposure (men)			
	<1 yr	14	0.8 (0.50-1.4)	96.6
	1-4.9 yr	25	1.2 (0.8-1.7)	98.4
	≥5 yr	29	1.6 (1.1-2.3)	97.0
	Duration of exposure (women)			
	<1 yr	2	1.1 (0.1-3.8)	30.1
	1-4.9 yr	3	1.2 (0.2-3.4)	37.1
	≥5 yr	3	1.5 (0.3-4.3)	31.9
Zhao et al. 2005	Kidney in men			
	Cumulative exposure score, lag 0			
	Low (0-3)	6	1	
	Medium (>3-15)	6	1.87 (0.56-6.20)	43.8
	High (>15)	4	4.90 (1.23-19.60)	18.7
Mortality				
Shindell and Ulrich 1985	White and nonwhite men and women in a manufacturing plant that used TCE as a degreasing agent	Not reported		
Garabrant et al. 1988	Aircraft manufacturing workers, San Diego (about 37% of jobs had exposure to TCE)	12	0.93 (0.48-1.64)	90.9
Costa et al. 1989	Aircraft and aerospace components workers, Turin, Italy	Not reported		
Boice et al. 1999	Aircraft manufacturing workers in California			
	Factory workers	75	0.86	100
	Routine exposure	7	0.99 (0.40-2.04)	71.3
	Duration of exposure to TCE			
	0	22	1	
	<1 yr	6	0.97 (0.37-2.50)	66.3
	1-4 yr	1	0.19 (0.02-1.42)	60.3
	≥5 yr	4	0.69 (0.32-2.12)	63.8

TABLE 3-5 *Continued*

Reference	Study Population	Exposed Cases	Estimated RR (95% CI)	Approximate Statistical Power to Detect a RR = 2
Blair et al. 1998	Male aircraft-maintenance workers in Utah			
	No TCE exposure	10	2.5 (0.7-8.9)	50.7
	<5 unit-yr	8	2.0 (0.5-7.6)	50.7
	5-25 unit-yr	1	0.4 (0.1-4.0)	37.1
	>25 unit-yr	4	1.2 (0.3-5.7)	44.9
	Frequent peaks	5	1.4 (0.3-5.7)	47.0
Morgan et al. 1998	Aerospace workers in Arizona, TCE exposed subcohort	8	1.32 (0.57-2.60)	65.5
	Any exposure	1	0.47 (0.01-2.62)	33.3
	Low exposure	7	1.78 (0.72-3.66)	50.1
	High exposure			
	Peak exposures (reference=none/low)			
	Medium/high	8	1.89 (0.05-4.3)	52.5
	Cumulative (low)	1	0.31 (0.04-2.36)	43.9
	Cumulative (high)	7	1.59 (0.68-3.71)	53.9
Henschler et al. 1995	Male German cardboard manufacturers, employed >1 yr	2	3.28 (0.40-11.84)	16.0
Greenland et al. 1994	White male U.S. transformer manufacturers, ever exposed to TCE	NA	0.99 (0.30-3.32)	
Ritz 1991	Mortality experience of male uranium-processing plant workers			
	Total cohort	8	1.17 (0.50-2.31)	70.1
	Bladder and kidney combined			
	No. of yr exposed			
	TCE level 1			
	<2 yr	6	1	
	2-10 yr	5	1.94 (0.59-6.44)	37,9
	≥10 yr	2	0.76 (0.14-400.0)	38.3
	TCE level 2			
	<2 yr	13	1	
	2-10 yr	0	0	
	≥10 yr	0	0	

TABLE 3-5 *Continued*

Reference	Study Population	Exposed Cases	Estimated RR (95% CI)	Approximate Statistical Power to Detect a RR = 2
Sinks et al. 1992	Cardboard manufacturers, USA			
	SMR	1	1.4 (0.0-7.7)	17.3
Chang et al. 2003	Kidney and other unspecified urinary organs			
	Men	0	0 (0.0-2.82)[a]	
	Women	3	1.18 (0.24-3.44)	37.5
	Men and women			
	Duration of employment			
	<1 yr	1	0.62 (0.02-3.46)[a]	27.8
	1-5 yr	2	3.08 (0.37-11.11)[a]	16.6
Zhao et al. 2005	Kidney in men			
	Cumulative exposure score, lag 0			
	Low (0-3)	7	1	
	Medium (>3-15)	7	1.43 (0.49-4.16)	57.6
	High (>15)	3	2.03 (0.50-8.32)	26.4

Abbreviations: CI, confidence interval; NA, not available; RR, relative risk; SMR, standardized mortality ratio.
[a]95% CI calculated by the committee using standard methods from the observed and expected numbers presented in the original study.
Source: Adapted from IOM 2003.

TABLE 3-6 Average Annual Incidence (per 100,000) of Kidney and Renal Pelvis Cancer in the United States[a]

	50–54 Years of Age			55–59 Years of Age			60–64 Years of Age		
	All Races	White	Black	All Races	White	Black	All Races	White	Black
Males	21.6	21.7	31.8	34.7	35.4	46.5	49.7	51.2	57.0
Females	10.1	10.4	12.7	16.3	16.9	20.6	22.8	23.6	29.8

[a] SEER (Surveillance, Epidemiology, and End Results program) 13 registries, crude age-specific rates, 1998-2002.
Source: SEER 2005.

that have low statistical power are not likely to provide useful evidence, unless somehow the results are combined, for or against the hypothesis that trichloroethylene is a human carcinogen.

The statistical power of a study is reflected in the estimated variances and the associated confidence intervals. As well, in meta-analyses the power of a study is effectively accounted for in the weight (inverse of the estimated variance) used to calculate the summary relative risk and to estimate whether there is heterogeneity in the rate ratios between studies. In qualitatively assessing studies, it is nevertheless useful to assess formally the statistical power of each study. Statistical power is a function of the true relative risks of the study population; if exposures in the population are low then relative risks will be smaller. Thus, plots of statistical power versus different values of relative risk can be produced. The usefulness of such plots is illustrated by Beaumont and Breslow (1981) in an analysis of cohort studies of workers exposed to vinyl chloride monomer. They described a useful method for presenting the data by plotting the power of detecting a relative risk for various values of relative risk on the ordinate and the expected number of deaths on the abscissa. An alternative formulation is to plot the observed relative risk and confidence intervals versus calculated statistical power.

For cohort studies that compare an exposed population with an unexposed population, an approximate formula for power is given by:

$$Z_{1-\beta} = Z_a - 2 \times \sqrt{E} \times \left(\sqrt{\mathrm{RR}} - 1\right),$$

where β is the Type II error
$Z_{1-\beta}$ is the Z value for power $1 - \beta$,
Z_a is the upper 100th percentile of the standard normal distribution,
E is the expected number of deaths, and
RR is the estimated rate ratio or relative risk.

For each of the cohort studies considered by the committee, Table 3-5 provides the approximate statistical power to detect a RR of 2 or more, on the basis of a statistical significance of 5% and the number of expected cases. (Power calculations could also be carried out for case-control studies.) Statistical power must be interpreted with knowledge of the exposure situation. For example, the study by Blair et al. (1998) had 82% power to detect RR values of 2, but the actual exposure in the cohort was so low that one would expect much smaller relative risks. However, the study did not have a large enough sample size to detect a smaller relative risk (the power to detect a RR of 1.5 was 39.6%). For this reason, plotting the power for various expected relative risks is useful. Even disregarding the degree of exposure, the power to detect excess risks was extremely limited in most studies. The essentially negative studies by Greenland et al. (1994), Axelson et al. (1994 [incidence]), Antilla et al. (1995 [incidence]), Blair et al. (1998 [incidence

and mortality]), Morgan et al. (1998), Boice et al. (1999), and Hansen et al. (2001) were all underpowered.

Effects of Confounding

The impact of confounding by other risk factors also needs to be considered carefully in evaluating the evidence for kidney cancer. In the cohort studies, potentially confounding factors other than accounting for age, sex, and calendar year were not evaluated. The major risk factors for kidney cancer appear to be cigarette smoking, obesity, and ionizing radiation; some evidence suggests that phenacetin-containing drugs, diuretics, and exposure to asbestos might be associated with incidence (McLaughlin et al. 1996). The RR values for current smokers are in the range 1.5-2.0 and, unless smoking was strongly associated with exposure to trichloroethylene, it is unlikely that smoking, or any other risk factors, could lead to substantial bias.

Take a hypothetical situation in which a cohort of subjects is exposed to trichloroethylene and the end point is incidence of kidney cancer. Assume that the observed standardized incidence ratio (SIR) is 2, but the prevalence of smoking in the cohort was not taken into account in its calculation, so that confounding could explain the observed effect. The following equation describes the relationship between incidence and exposure and the confounding effect of smoking:

$$I = I_0 \times \sum_{\iota} \left\{ 1 + p_{s,i} \times (\mathrm{RR}_{s,i} - 1) \right\}$$

where I is the incidence of kidney cancer, I_0 represents the baseline rate, $\mathrm{RR}_{s,i}$ represents the rate ratio for smoking (s) at level i (e.g., moderate, heavy), and $p_{s,i}$ is the proportion of individuals smoking at level i.

Given some realistic values of the proportion of workers smoking at different intensities and the relative risk of developing kidney cancer, the amount of bias can be calculated. Assume, for example, that there is no real association between kidney cancer and exposure to trichloroethylene, and only smoking increases the baseline incidence rate so that the RR for smoking is 2. Thus, the above equation reduces to $I = I_0 + p_c$. So, for example, if 50% of the population in the cohort smokes and 30% of the general population smokes, then the expected RR due only to smoking is 1.15, and this would not explain the observed RR of 2.

In most of these studies (Blair et al. 1998, Morgan et al. 1998, Boice et al. 1999, Ritz 1999), general population rates of kidney cancer were used as the comparison group, thereby indirectly standardized mortality ratios (SMR) or SIR were calculated. Such use of the general population as the reference population in most cases will lead to attenuated rate ratios because the workforce is generally healthier than the general population. This type of selection bias is one component of the "healthy worker effect" (Fox and Collier 1976; Choi 1992; Li and Sung 1999; Baillargeon 2001). Rate ratios estimated from internal comparisons (e.g., comparing an exposed group with an unexposed group within the study population) will not have such a bias, although there can be effects from selection out of the study. There is no simple method to estimate the extent of the latter type of bias (for advanced methods, see Robins 1986, 1987a,b,c).

Exposure Assessments

Table 3-7 presents essential characteristics of the exposure assessments for selected cohort studies. Important factors in these studies that affect causal inference is whether subjects were actually exposed to trichloroethylene, whether exposures were to complex mixtures, whether the measurements were accurate (validity, reliability), and whether exposure-response relationships were estimated. For some studies, whether subjects were actually exposed to trichloroethylene is doubtful (Shindell and Ulrich 1985; Garabrant et al. 1988; Sinks et al. 1992; Greenland et al. 1994). In some studies, biological monitoring for urinary trichloroacetic acid was conducted. The half-life of urinary trichloroacetic acid is on the order of 100 hours (Muller et al. 1974) and it represents total acute exposures (occupational and other sources) to trichloroethylene, tetrachloroethylene, and trichloroacetic acid in the environment (e.g., drinking water). In any risk assessment, close attention must be paid to the validity and reliability of the exposure assessments in all the included studies.

As indicated above, there is good reason to exclude all the dry cleaning studies because there was apparently little use of trichloroethylene (Stewart and Dosemeci 2005). In addition, the cohort study of Swedish Airforce personnel exposed to aircraft fuel (Selden and Ahlborg 1991) should be excluded, as it is likely that they were exposed to no more than trace amounts of trichloroethylene.

Specific Issues with Certain Cohort Studies

Studies from Arnsberg Area of Germany. Considerable controversy has surrounded the studies by Henschler et al. (1995) and Vamvakas et al. (1998) conducted in the Arnsberg area of Germany. Recent papers by Brüning et al. (2003) and Pesch et al. (2000a) cover the same region in Germany but at a later time. The region was a center of metal machining and processing operations. This concentration of operations resulted in a high prevalence of workers with intense exposures to trichloroethylene.

The finding of a cluster of cases prompted the study by Henschler et al. (1995). Because the study population included the area with the cluster, the observed relative risks for kidney cancer were much higher than in any other study (SMR, 7.97; 95% CI, 2.59-18.59; five cases), compared with incidence rates from the Danish Cancer Registry. Because of its very high SMR, this study has been the subject of much scrutiny. The study population comprised a cohort of 169 workers reportedly exposed to trichloroethylene at a cardboard manufacturing plant. The workers were employed for at least 1 year between 1956 and 1975. The comparison populations comprised a matched cohort of 190 workers from the plant who were not exposed to trichloroethylene as well as the general population of Denmark and Germany, as reflected by rates of cancer incidence from the Danish Cancer Registry and mortality from a German registry. Follow-up of both populations was from 1956 until the end of 1992. Trichloroethylene was used in degreasing operations from 1956 to 1975 and no specific exposure assessments were carried

TABLE 3-7 Characteristics of the Assessment of Exposure to Trichloroethylene in Selected Cohort Studies

Study	Qualitative Assessment (industry, TCE use, coexposures, exposure prevalence X%, confounders)	Information on Settings (location of exposures, area descriptors, jobs, tasks, exposure controls)	Duration (source, data quality, period, duration of exposure, sufficient latency)	Exposure Quantification (methods, relative errors, range over time & categories, prevalence of high exposures)	Exposure Extrapolation (methods, assumptions, data sources [direct, indirect])	Dose Metrics (quantitative, semiquantitative, qualitative)
Biomonitored populations						
Antilla et al. 1995 (2,050 males and 1,924 subjects ever monitored)	Finnish mixed industries using TCE in 1950s and 1960s (also Perc and TCA).	Limited general description; most people tested were degreasing or metal cleaners.	About 1930-1982 In cohort 1967, mean follow-up was 18 yr; time since first test was assumed to be exposure duration.	U-TCA	None (Using the Ikeda [1972] relationship for TCE exposure to U-TCA, median exposures were <4-9 ppm.)	Biomarker values
Axelson et al. 1994 (1,421 males and subjects ever monitored)	Swedish mixed industries using TCE.	Limited general description.	About 1930-1986 In cohort 1955, time since first test was assumed to be exposure duration.	U-TCA	None (Using the Ikeda [1972] relationship for TCE exposure to U- TCA, median exposures were <20 ppm.)	Biomarker values
Hansen et al. 2001 (803 males and female subjects ever monitored)	Danish mixed industries using TCE, predominantly iron and metal product producers.	Limited general description.	Retirement records and job records from 1947 to 1989.	U-TCA	None (Using the Ikeda [1972] relationship for TCE exposure to U-TCA, median exposures were <2-7 ppm.)	Biomarker values

TABLE 3-7 *Continued*

Study	Qualitative Assessment	Information on the Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Aircraft workers						
Stewart et al. 1991 (exposure assessment); Spritas et al. 1991 (epidemiologic analysis; cohort 14,457) Blair et al. 1998 (epidemiologic analysis for reevaluation of cohort)	Aircraft maintenance; solvents used for degreasing and cleaning; carbon tetrachloride until mid-1950s; TCE used up to 1970s: historical changes: about 1955-1978 TCE used in vapor degreasing; about 1955-1968 TCE used in hand degreasing; before 1955 Stoddard and carbon tetrachloride were used; about 50% exposed to TCE; coexposures to Stoddard solvent/ kerosene, 1,1,1-trichloroethane, gasoline, jet fuel, isopropyl alcohol; about 65% of men and 33% of women were exposed to TCE.	Large maintenance facility at Hill Air Force Base, UT. Detailed records on setting and job activities, worker interviews; work done in large open shops; shops not recorded in personnel records; link of jobs with exposures was weak.	From payroll records, 1940s-1990. In cohort if >1 yr in 1952-1956. Total duration likely to be accurate, but duration of specific exposures uncertain because of weak linkage of jobs and exposures; about 35 yr latency possible; exposures noted after 1951.	Limited exposure monitoring data 1960-1990 for TCE. Plant JEM, rank order assignments by history; determined exposure duration during vapor degreasing tasks about 200 ppm/hr and hand degreasing about 20 ppm/hr; only 16% of total deaths occurred in subjects with >25 unit-yr (no data given on number of subjects with long high exposures).	Similar jobs had similar exposures; limited data for early periods; exposure data for shops, but jobs in histories not linked to shops. Large misclassification of exposures in mixed solvent group. Median exposures were about 10 ppm for rag and bucket; 100-200 ppm for vapor degreasing.	Cumulative exposure; calculated but results not reported; average exposure and duration of exposure.

TABLE 3-7 *Continued*

Study	Qualitative Assessment	Information on the Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Boice et al. 1999 (cohort 77,965)	Aircraft manufacturing; four different sites in Burbank, CA; TCE used 1960-1970s; 12% with TCE exposure (5,443); coexposures to other chlorinated solvents, ketones, alcohols, petroleum distillates, chromate, paints, cutting fluids, and fibers.	Very limited on work places; jobs in work histories collapsed into groups with "similar" exposures.	Company records; good quality; provided data on start and end dates; median durations, exposure about 10 yr, latency about 30 yr.	Workers assigned to families: assembly, fabrication, processing, maintenance; smaller groups: processing operators and helpers (1,440) worked on vapor degreasers.	None	Years TCE exposure as routine or intermittent; job type and duration of exposure.
Morgan et al. 1998 (cohort 20,508)	Aerospace manufacturing but no details given on jobs or operations, or other exposures; knowledgeable employees assisted by company industrial hygienist, identified when and where TCE was used; degreasers about 1952-1977; about 23% were exposed to TCE.	Very limited; no data on buildings or operations; unclear if information on setting was used.	Company records on each subject provided hire and end dates; two-thirds had >20 yr latency; limited data on duration of exposures, none on high exposures.	No detail on protocol or possible errors; exposure range is uncertain; three categories assigned: high, work on degreasers (>50 ppm); medium, work near degreasers; low, occasionally near degreasers; no data on prevalence of high exposures.	Long-term employees rated exposures by vague criteria; company industrial hygienist used the data to build an exposure matrix; no consideration of changes with time.	Cumulative exposure by TCE weight times months; exposed to peaks was any time in high category; also a dichotomy of ever exposed, yes or no.
Costa et al. 1989 (cohort 8,626)	Aircraft manufacturing; many coexposures listed. TCE used up to 1970s; prevalence of TCE exposure not reported; many chemicals used over the years but not linked to subjects.	One aircraft manufacturing site in Italy; no data on the site were given.	Personnel records >1954; quality good; yr started and ended for each subject; median durations: employment about 10 yr, follow-up about 17 yr, only 25% had >25 yr.	None; used only four broad job groups: blue collar 75% of total, technical staff, administrative clerks, and white collar.	None	Job groups

TABLE 3-7 *Continued*

Study	Qualitative Assessment	Information on the Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Garabrant et al. 1988 (cohort 14,067)	Aircraft manufacturing; TCE usage not described; plant with detailed records on small sample, and interviews, assigned about 37% TCE exposed TCE used up to 1970s.	Major process categories listed with percentage of subjects in each from a sample of work histories.	Work records, quality good; >4 yr work Latency <30 yr; mean follow up was 15.8 yr	None for the cohort	None, no link with jobs or exposures.	Duration of employment
Mixed settings						
Raaschou-Nielsen et al. 2003 (exposures in Raaschou-Nielsen et al. 2002)	Mixed companies	Limited general description from national sources.	In cohort 1952	Samples from national program; iron and metal: quantitation not used in mortality study.	Regression relationship was developed by industry. GM: 60 ppm 1947-1959; 49 ppm 1960-1969; 20 ppm 1970-1979	
Ritz 1999	Uranium-processing workers using TCE in 1950s and 1960s, also cutting fluids, kerosene, company records.		>3 yr, 1951-1996 In cohort 1951	Exposure monitoring data, plant JEM, semi-quantitative assignments by industrial hygienists and employees.	None	
Chang et al. 2003	Large electronics plant; used TCE and Perc (found in local well water).	Taiwan; no direct information was provided on presumed TCE and Perc exposures.	Short duration about 10 yr	None	None	Years of employment
Sinks et al. 1992	Paperboard manufacturing, TCE present in materials, usage not described.	TCE usage not described.	Work histories, quality good, duration in depts.; sufficient latency.	None	None	Duration in department

TABLE 3-7 *Continued*

Study	Qualitative Assessment	Information on the Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Henschler et al. 1995	Cardboard manufacturer in Arnsberg, Germany. TCE used as degreasing solvent, small amounts of TCA and Perc also used.	Highly detailed on TCE use, area descriptors (hot machines and areas, large amount of TCE used, jobs, tasks) extensive cleaning; no controls.	Company data, questionnaire, interviews, median 34 yr observation, sufficient latency.	None (neurological symptoms reported, >200 ppm likely).	None in paper (Cherrie et al. [2001] extrapolated based on details in paper: peaks >2,000 ppm long-term 100-200 ppm).	Exposed vs. unexposed

Abbreviations: JEM, job exposure matrix; Perc, tetrachloroethylene; TCA, trichloroacetic acid; TCE, trichloroethylene; U-TCA, urinary metabolite of trichloroethylene (and Perc, trichloroethane, and 1,1,1-trichlorethane).

out. However, extensive detail about the nature of the operations permitted extrapolations of possible exposures (Cherrie et al. (2001).

The Henschler et al. (1995) study has been criticized for a number of reasons, including the following:

1. A cluster of cases of kidney cancer were used to design the study, and they were included in the analysis (see Swaen 1995; McLaughlin and Blot 1997). As a consequence, statistical inference is not valid unless the cluster is removed, but removing the cluster would reduce substantially the study's statistical power. An additional consequence of having an incorrect estimate of statistical precision is that the study could not be included in a formal meta-analysis and care must be taken in interpreting risk estimates in informal analyses. Although estimates of rates and relative rates are valid, the main issue is how to calculate an appropriate variance so that statistical tests can be conducted and confidence intervals estimated correctly. The issue is that the nominal *P* values do not apply as this working population was selected because of the cluster. The option of excluding the cluster of cases could be implemented if the cluster occurred at the beginning of the risk period and did not include all cases; then theses cases and person-time could be excluded, yielding a valid estimate of risk and statistical precision. McLaughlin and Blot (1997) suggested that the first three cases diagnosed in 1990 and 1991 might represent the original cluster; in that case, the SMR would be approximately 3.2.

2. Fourteen subjects who were lost to follow-up were excluded, although they should have been included in the person-year calculations (Swaen 1995). The likely effect of this exclusion is that the number of person-years is underestimated; the expected number of deaths is less than it should be, so the observed risk ratio is overestimated. The committee concludes that, given the small number of subjects, the extent of underestimation is likely not great.

3. Different methods were used to assign underlying causes of death in the cohort and reference population (Swaen 1995). This could have introduced a positive bias (away from the null) if the hospital or physician records were more likely to detect cases of the cancers of interest than the medical institutions serving the general population. The committee concluded that this effect could not have explained the entire excess risk.

4. The follow-up of some subjects might have been outside the range of the stated follow-up period (Bloemen and Tomenson 1995). The committee did consider this a pertinent criticism, as it would have resulted in an overestimation of person-time and expected deaths and thereby would have led to an underestimate of the relative risks.

5. The intensity of exposure to trichloroethylene in this occupational environment was unclear (Swaen 1995; Cherrie et al. 2001). However, given the information the paper provided on the job activities and work locations, the committee judges that the magnitude of the exposures can approximated. Cherrie et al. (2001) extrapolated the exposures on the basis of the data presented and a simple engineering model of the workplace. The analysis indicated that the subjects in this study likely had substantial peak exposures to trichloroethylene above 2,000 ppm and probably had sustained long-term exposures above 100 ppm.

6. Use of the Danish Cancer Registry to calculate expected values might not be appropriate if rates of renal cell cancer in the region surrounding the plant are very different from those in Denmark. The age-standardized rate in the late 1990s among men in Denmark was 10.6 and in Germany it was 12.3 (Ferlay et al. 2004). If these differences in rates apply when the study was carried out, this would imply that the expected number of deaths would have been inflated by about 14% (and the rate ratio underestimated by that amount).

7. Could there be unmeasured confounding? The low SMR for lung cancer (mortality SMR = 1.4 in the exposed cohort) suggests that it is unlikely that smoking would play an important role.

Although clear methodological difficulties are associated with this study, which leaves doubt about the veracity of the results, if exposure to trichloroethylene were as high as suggested (Henschler et al. 1995) then part of the excess could reflect a causal process at high exposures. The committee concludes that it is neither prudent nor useful to ignore the findings of this study, but they must be evaluated within the context of the available literature, particularly in the context of other studies from the same base population.

New Studies Published in 2005. The committee reviewed one report that was published during its deliberations (Zhao et al. 2005). This investigation was a cohort study of 6,107 male workers at the Santa Susana Field Laboratory (Boeing) in California, employed between 1950 and 1992. The follow-up period was until the end of 2001. This study is one of the few to conduct a detailed assessment of exposure that allowed for the development of a job-exposure matrix that provided rank-ordered levels of exposure to trichloroethylene, benzene, polycyclic aromatic hydrocarbons, mineral oil, and hydrazine. A monotonic increase in mortality rates due to kidney cancer by increasing levels of cumulative exposure was found. This relationship was accentuated with adjustment for other occupational exposures, although the confidence intervals were increased. This phenomenon is not unusual in occupational studies as exposures are usually highly correlated and adjustments often inflate standard errors without removing any bias. Thus, these adjustments must be interpreted cautiously.

Case-Control Studies

The main methodological points associated with case-control studies are the accuracy of the outcome, estimates of exposure, statistical power, confounding, and selection bias. Tables 3-8 and 3-9 provide the essential design characteristics and selected results of case-control studies on kidney cancer and occupational exposure to trichloroethylene. Each of these studies would need to be evaluated more fully as to the likely level of exposure and the proportion of subjects exposed to trichloroethylene. In particular, this is necessary for the pooled analysis by Mandel et al. (1995), in which only occupations were reported (based on studies by McCredie and Stewart [1993], Schlehofer et al. [1995], and others). Including estimates of statistical power for the case-control studies, as was done in Table 3-5, might be useful.

In contradistinction to the cohort studies, in most of the case-control studies the diagnosis of cancer was confirmed histologically (except, perhaps, in the studies by Ashengrau et al. [1993] and McCredie and Stewart [1993]). Thus, few subjects who did not have a tumor would be included. In addition, many of these studies had a large number of cases (e.g., more than 200) and, if the prevalence of exposure to trichloroethylene were sufficiently high, then there would be adequate power to detect positive associations should trichloroethylene be a risk factor for kidney cancer. However, a number of small case-control studies lacked the statistical power to detect modest associations with exposure to trichloroethylene (Jensen et al. 1988; Harrington et al. 1989; Sharpe et al. 1989; Aupérin et al. 1994; Vamvakas et al. 1998; Parent et al. 2000; and

TABLE 3-8 Description of Case-Control Studies That Present Associations Between Kidney Cancer and Possible Exposure to Trichloroethylene

Reference	Description of Study Population	Number of Cases	Number of Controls	Relevant Exposure(s)	Determination of Exposure	Analysis	Adjustment for Confounding
Asal et al. 1988	Cases from 29 hospitals in Oklahoma diagnosed and confirmed in 1981-1984; hospital controls selected from the same hospitals and matched on age, sex, race, hospital, and date of admission; population-based controls selected through RDD. No response rates provided.	315 renal cell carcinoma	313 hospital 336 population	Dry cleaning work Painter or paint-manufacturing work	In-person interview assessing occupations (job titles) and industrial exposures.	Logistic regression	Weight, age, alcohol consumption, occupation, smoking, snuff use, coffee consumption, kidney stones, hypertension, other medical factors
Jensen et al. 1988	Cases, under age 80 yr, reported to the Danish Cancer Registry from Copenhagen and the surrounding island of Sjaelland in 1979-1982, with 90% histologic verification; controls selected from hospital where cases arose, excluding those with urinary tract and smoking-related diseases; controls matched for hospital, sex, and age. Response rates: 99.0% of cases, 100.0% of controls.	96 renal, pelvis, and ureter	288	Painter or paint-manufacturing work	In-person interviews with questionnaire assessing personal habits and occupational history (job or industry titles and self-reported exposures).	Logistic regression Although controls were matched to cases (ratio 3:1) on hospital, age, and sex, a matached analysis was not conducted.	Sex, lifetime tobacco smoking

TABLE 3-8 *Continued*

Reference	Description of Study Population	Number of Cases	Number of Controls	Relevant Exposure(s)	Determination of Exposure	Analysis	Adjustment for Confounding
Harrington et al. 1989	Cases diagnosed and histologically confirmed in 1984-1985 and reported to the West Midlands Regional Cancer Registry (UK); controls randomly selected from practitioner records and matched for age, sex, ethnicity, location, and socioeconomic group. No response rates provided.	54 renal (adeno-carcinoma)	54	Solvents	In-person interviews with questionnaire assessing lifetime occupational history (job titles); exposure indexes determined by occupational hygienist or chemist, but not defined explicitly Values used: "unexposed," index <1; "intermediate," 1-99; "exposed," >100.	Matched analyses	Matching variables
Sharpe et al. 1989	Cases diagnosed at one of four Montreal-area hospitals in 1982-1986 and one of five other hospitals in 1982-1987; cases were histologically confirmed and alive at time of chart review; controls selected from suspected renal cell carcinoma cases, but final diagnoses were not cancer; matched 1:1 for sex, age (+5 yr), and urologist. Response rate: 97% overall.	164 renal prevalent cases	161	Organic solvents	History of exposure to hydrocarbons obtained through mailed questionnaire and supplemented by telephone interview (self-reports).	Unadjusted analysis, but matched-unadjusted	None
Partanen et al. 1991	Cases, age over 20 yr, identified through the Finnish Cancer Registry in 1977-1978; controls randomly selected from the Population Register Centre matched for year of birth, sex, and survival status. Excluded subjects who did not work. Response rates: 69% of cases, 68% of controls.	408 renal cell	819	Nonchlori-nated solvents	Mailed questionnaire or phone interview (direct or proxy) assessing lifetime occupational history (job or industry titles); industrial hygienist coded and assigned summary indicators of specific exposures.	Conditional logistic regression	Matching variables, smoking, coffee consumption, obesity

TABLE 3-8 *Continued*

Reference	Description of Study Population	Number of Cases	Number of Controls	Relevant Exposure(s)	Determination of Exposure	Analysis	Adjustment for Confounding
Aschengrau et al. 1993	Cases reported to the Massachusetts Cancer Registry, diagnosed in 1983-1986 among residents of five upper Cape Cod towns; living controls were selected from HCFA records and RDD; deceased controls identified by the state Department of Vital Statistics and Research files. Response rates: 80.6% of cases, 75.9% of HCFA controls, 73.9% of RDD controls, 78.8% of next of kin of deceased controls.	35 kidney	777	Tetrachloro-ethylene	Exposure dose estimated in areas of contaminated drinking water, accounting for location and years of residence, water flow, pipe characteristics.	Logistic regression	Sex, age at diagnosis, vital status, educational level, usual number of cigarettes smoked, occupational exposure to solvents, specific cancer risk factors controlled for in respective analyses.
McCredie and Stewart 1993	Cases, age 20-79 years, among residents of New South Wales in 1989-1990 identified from the New South Wales Central Cancer Registry and from physicians; controls selected from electoral rolls and matched on age distribution. Response rates: 68% renal cell cases; 74% renal pelvis cases; 74% controls. Included in the combined analysis of Mandel et al. 1995	489 renal cell 147 renal pelvic	523	Dry-cleaning industry work, solvents	Questionnaire (in-person interview or mailed with telephone follow-up) to assess employment in specific occupations and industries (job or industry titles), self-reported.	Logistic regression	Age, sex, interview method, cigarette smoking, body mass index, education, analgesic use.

TABLE 3-8 *Continued*

Reference	Description of Study Population	Number of Cases	Number of Controls	Relevant Exposure(s)	Determination of Exposure	Analysis	Adjustment for Confounding
Aupérin et al. 1994	Cases of renal cell carcinoma diagnosed in France, 1987-1991, confirmed histologically. Controls matched by age, sex, hospital, and interviewer and comprised nonmalignant and malignant disease. Response: about 100%	196 cases (138 men, 58 women)	347 161 cancer 186 other diseases	Occupation or industry	In-person interview in hospital, occupational history.	Conditional logistic	Matching variables, education, smoking, body mass index.
Mellemgaard et al. 1994	Cases, age 20-79 years, identified from the Danish Cancer Registry and pathology departments in 1989-1992 with histologic confirmation; controls selected from the Central Population Register and matched for age and sex. Response rates: 80% of cases, 79.2% of controls.	368 renal cell	396	Dry cleaning work, solvents	In-person interviews with questionnaire assessing most recent and longest-held occupation (job titles) and exposure to specific agents (self-reports).	Logistic regression	Age, body mass index, smoking.
Mandel et al. 1995	Cases, age 20-79 years, from six international sites, diagnosed and confirmed in 1989-1991 using cancer registries or surveillance of clinical and pathology departments; controls selected from population registers, electoral rolls, residential lists, HCFA records, or RDD, depending on the site; controls matched on age and sex. No response rates provided (included studies of McCredie and Stewart 1993 and Schlehofer et al. 1995).	1,732 renal	2,309	Occupational titles, including dry cleaning solvents, dry cleaning work	In-person interviews to assess lifetime occupational history (job titles) and exposure to specific agents (self-reports).	Logistic regression	Age, center, body mass index, cigarette smoking.

TABLE 3-8 *Continued*

Reference	Description of Study Population	Number of Cases	Number of Controls	Relevant Exposure(s)	Determination of Exposure	Analysis	Adjustment for Confounding
Schlehofer et al. 1995	Cases, age 20-75 years, identified through 10 urology departments in the Rhein-Neckar-Odenwald area of Germany in 1989-1991 with histologic confirmation; controls randomly selected from population register and matched on age and sex. Response rates: 84.5% of cases, 75% of controls. Included in combined analysis of Mandel et al. 1995	277 renal cell	286	Chlorinated solvents	In-person interview with questionnaire assessing exposure (in excess of 5 years) from list of specific substances (self-reports).	Logistic regression	
Parent et al. 2000; Siemiatycki 1991	Male cases, age 35-75 years, diagnosed in 1 of 19 large Montreal-area hospitals in 1979-1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls were also chosen from electoral lists and RDD. Response rates: 82% of all cases, 71% of population controls.	142 renal cell	2433, consisting of 533 population controls and 1,900 subjects from other cases of cancer.	294 agents, including TCE. Results for TCE not published, but obtained from authors.	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (about 300 exposures on semi-quantitative scales).	Logistic regression	Age, body mass index, cigarette smoking, respondent status.

TABLE 3-8 *Continued*

Reference	Description of Study Population	Number of Cases	Number of Controls	Relevant Exposure(s)	Determination of Exposure	Analysis	Adjustment for Confounding
Vamvakas et al. 1998	Cases who underwent nephrectomy in 1987-1992 in a German hospital; controls selected in 1993 from accident wards of three nearby hospitals. Response rates: 79.5% of cases, 75% of controls.	58 renal	84	Trichloro-ethylene	In-person, unblinded interviews by physicians (direct or proxy) with structured questionnaire assessing occupational history (job titles) and specific agent exposures (self-reports). Ranking of exposures based on assessment of duration, frequency, concentration, and prenarcotic symptoms. Not clear how exposure was assessed.	Logistic regression	Age, sex, smoking, body mass index, blood pressure, intake of diuretics.
Chow et al. 1994; Dosemeci et al. 1999	White cases, age 20-85 years, with histologically confirmed diagnosis identified through the Minnesota Cancer Surveillance System in 1988-1990; controls identified through RDD (age 21-64 years) and HCFA records (age 65-85 years), stratified for age and sex. Response rates: 87% of cases, 86% of controls. Occupational analysis: 55% of cases, 83.6% of controls.	438 renal cell carcinoma	687	Trichloroethylene, tetrachloro-ethylene, solvents in general.	In-person interviews (direct or proxy) with questionnaire assessing occupational history; job titles were coded and merged with a job-exposure matrix from the National Cancer Institute.	Logistic regression	Age, smoking, hypertension, body mass index.

TABLE 3-8 *Continued*

Reference	Description of Study Population	Number of Cases	Number of Controls	Relevant Exposure(s)	Determination of Exposure	Analysis	Adjustment for Confounding
Pesch et al. 2000a	Cases in large hospitals in five regions in Germany in 1991-1995 with histologic confirmation; controls randomly selected from local residency registries matched on region, sex, and age. Response rates: 88% of cases, 71% of controls.	935 renal cell	4,298	Trichloro-ethylene, tetrachloro-ethylene, organic solvents.	In-person interviews of lifetime occupational history using questionnaire to assess job titles and self-reported exposures, exposures ascertained by job-exposure matrices.	Conditional logistic regression	Matching variables, smoking.
Brüning et al. 2004	Cases of renal cell carcinoma diagnosed in Germany (same region as in Vamvakas and Henschler studies), 1992-2000, and study conducted in 1999-2000. Controls, matched by sex and age (+5 yr), selected from surgery wards and departments of geriatrics in same area. Response rates: 82.7% of cases, not stated for controls.	134 prevalent cases	401	Occupation or industry, specific exposure from JEM, self-reported exposure.	Occupational history and specific to TCE, self-reports of exposure to TCE and perchloroethylene, reports of prenarcotic symptoms for peak exposures. Used job-exposure matrix of Pannett and the CAREX system to infer exposures.	Conditional logistic (based on frequency-matched)	Matching variables, smoking.

Abbreviations: HCFA, health care financing administration; JEM, job exposure matrix; RDD, random digit dialing; TCE, trichloroethylene.
Source: Adapted from IOM 2003.

TABLE 3-9 Selected Results from Case-Control Studies of Kidney Cancer and Occupational Exposure to Trichloroethylene

Reference	Study Population	Exposed Cases	Estimated RR (95% CI)
Siemiatycki, 1991; Parent et al. 2000	Kidney cancer, men, Montreal Exposure assessed by experts from job descriptions		
	Any exposure	4	0.8 (0.4-2.0)[a]
	Substantial exposure	2	0.8 (0.2-2.6)[a]
Dosemeci et al. 1999	Ever exposed to TCE, according to National Cancer Institute job-exposure matrix	55	1.30 (0.9-1.9)
	Residents of Minnesota	33	1.04 (0.6-1.7)
	Men	22	1.96 (1.0-4.0)
	Women		
Vamvakas et al. 1998	Residents of Germany with long-term exposure	19	10.8 (3.36-34.75)
Pesch et al. 2000a	Participants in multiple centers in Germany German job-exposure matrix		
	Trichloroethylene (men)		
	Medium	135	1.1 (0.9-1.4)
	High	138	1.1 (0.9-1.4)
	Substantial	55	1.3 (0.9-1.8)
	Trichloroethylene (women)		
	Medium	28	1.2 (0.8-1.8)
	High	29	1.3 (0.8-2.0)
	Substantial	6	0.8 (0.3-1.9)
	Job task-exposure matrix approach Trichloroethylene (men)		
	Medium	68	1.3 (1.0-1.8)
	High	59	1.1 (0.8-1.5)
	Substantial	22	1.3 (0.8-2.1)
	Trichloroethylene (women)		
	Medium	11	1.3 (0.7-2.6)
	High	7	0.8 (0.4-1.9)
	Substantial	5	1.8 (0.6-5.0)

TABLE 3-9 *Continued*

Reference	Study Population	Exposed Cases	Estimated RR (95% CI)
Brüning et al. 2004	Longest-held job (men and women)		
	Trichloroethylene and perchloroethylene	117	1.80 (1.01-3.20)
	Ever employed		
	Metal greasing and degreasing	15	5.57 (2.33-13.32)
	Metal processing	30	1.34 (0.81-2.23)
	Metalworking	9	2.33 (0.91-5.94)
	Pannett job-exposure matrix		
	Degreasing agents		
	Low	9	2.11 (0.86-5.18)
	High	7	1.01 (0.40-2.54)
	Solvents		
	Low	8	1.80 (0.70-4.59)
	High	8	1.45 (0.59-3.58)
	Self-reported exposure		
	Trichloroethylene	25	2.47 (1.36-4.49)
	Perchloroethylene	7	1.64 (0.61-4.40)
	Solvents	36	2.55 (1.41-4.35)
	Self-reported narcotic symptoms		
	Trichloroethylene	19	3.71 (1.80-7.54)
	Perchloroethylene	5	1.84 (0.57-5.96)
	Duration of self-reported exposure to TCE		
	None		
	<10 yr	109	1
	10-20 yr	11	3.78 (1.54-9.28)
	>20 yr	7	1.80 (0.67-4.79)
		6	2.69 (0.84-8.66)

Abbreviations: CI, confidence interval; RR, relative risk; TCE, trichloroethylene.
[a]90% CI.
Source: Adapted from IOM 2003.

Brüning et al. 2003). Graphs similar to that suggested earlier for cohort studies of effect versus power could be developed for these case-control studies (see Beaumont and Breslow 1981). Calculations of power for case-control studies have been published (Self and Mauritsen 1988; Self et al. 1992) and are available in some software packages (e.g., Egret SIZ).

Confounding

As discussed in the section on cohort studies, risk factors for kidney cancer include cigarette smoking, obesity, and ionizing radiation; phenacetin-containing drugs, diuretics, and exposure to asbestos may also be associated with incidence of kidney cancer (McLaughlin et al.

1996). Studies by Asal et al. (1988), Partanen et al. (1991), McCredie and Stewart (1993), Auperin et al. (1994), Chow et al. (1994), Mellemgaard et al. (1994), Mandel et al. (1995), Vamvakas et al. (1998), and Parent et al. (2000) controlled for at least smoking and body mass index. Because it is unlikely that exposure to trichloroethylene is associated with these factors, the committee judges that they do not significantly affect the estimates of risk. However, it might be useful in the risk assessment to distinguish and compare estimates of risk between studies in which confounding was and was not accounted for.

One possible source of bias that affects all case-control studies to some degree is the nonrepresentativeness of the study population to the target population. This can occur through sampling variation (essentially a random error, which is reflected in the estimates of variance and confidence intervals) and also through systematic effects in which subjects are recruited in a nonrandom way that might depend on exposure status (selection bias). In most case-control studies, the sampling fraction for cases is close to 100%, so that any selection biases would be manifest only if selection probabilities in the control series varied with exposure. However, low response rates can affect the validity of the findings, especially if they are also associated with exposure. The committee did not inspect the case-control studies for possible selection biases, but this is a necessary step in the risk assessment. In addition, the committee did not evaluate every study with regard to response rates, except noting low response rates in some studies (e.g., Partanen et al. 1991; McCredie and Stewart 1993) and those studies in which response rates were not stated (Asal et al. 1988; Harrington et al. 1989; Mandel et al. 1995).

Exposure Assessment

Table 3-10 presents the essential characteristics of exposure for selected case-control studies. The committee has general concerns about studies that use self-reports of exposures to occupational agents, especially in large urban centers where the prevalence of any one exposure would be low (Sharpe et al. 1989; Mandel et al. 1995; Schlehofer et al. 1995). In contrast to personal characteristics, such as age and race, for which subjects' reports are the gold standard, most individuals do not know what agents they used, especially if the agent occurred as part of a mixture or if a wide range of materials were used. On the other hand, most workers probably would know if they used a degreasing solvent.

The committee recognizes that there are special situations in which subjects are aware of their exposure circumstances. Questions about observable, objective facts tend to be answered accurately, such as "was there visible smoke in the air?" In workplaces where one or two materials are widely used, workers often know their common names, such as TCE, "Perc" (tetrachloroethylene), or Stoddard solvent, because there are limited choices and it makes a difference in the workplace which one is used. It is likely that workers enrolled in the study by Vamvakas et al. (1998) knew what solvent they were using; trichloroethylene was the solvent of choice for degreasing because it evaporates rapidly.

As with the cohort studies, an important factor that affects causal inference is how exposure is measured and assigned (validity, reliability) and whether exposure-response relationships are measurable. In studies in which analyses were conducted only by job title (Asal et al. 1988; Jensen et al. 1988; McCredie and Stewart 1993; Aupérin et al. 1994; Mellemgaard et al. 1994; Mandel et al. 1995), there would have been substantial misclassification that would have attenuated rate ratios. In addition, in some studies exposure was attributed by expert

TABLE 3-10 Characteristics of the Assessment of Exposure to Trichloroethylene in Selected Case-Control Studies

Study	Qualitative Assessment	Information on Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Vamvakas et al. 1998 (follow-up to Henschler study) (pending legal cases for workers' compensation—possible bias?)	Small companies making metal parts in Arnsberg area (no cases from Henschler case group); TCE used for cleaning; high prevalence of companies in area; some use of Perc; qualitative assessment by occupational hygienists and physicians, interviews.	Detailed info on TCE use, area descriptors—hot dip baths, small work areas, jobs, tasks—extensive cleaning; no controls.	Questionnaire, interviews, median 33 yr observation, sufficient latency.	None (frequency and severity of neurological symptom reports used to estimate high exposures; >200 ppm likely).	None in paper (Cherrie et al. [2001] extrapolated based on details in paper: peaks 400-600 ppm, long-term about 100 ppm).	Combination of symptom severity and frequency and duration of exposures ranked +, low; ++, medium; +++, high.
Bruning et al. 2003 (follow-up to Vamvakas study)	Small companies making metal parts in Arnsberg area (no cases from Vamvakas case group); TCE used for cleaning; high prevalence of companies in area; some use of Perc; qualitative assessment by occupational hygienists.	Detailed info on TCE use, area descriptors—hot dip baths, and small work areas, jobs, tasks—extensive cleaning; no controls.	Questionnaire, interview job histories and next of kin; median 33 yr observation, sufficient latency.	None (self-reported exposures, and two JEMs, CAREX, and British; >200 ppm likely for symptom reports).	None in paper (Cherrie et al. [2001] extrapolated based on details in paper: peaks 400-600 ppm, long-term about 100 ppm).	Job and industry groups with exposures from JEM; self-reported exposure and symptom frequency.
Brauch et al 1999; 2004 (molecular study of von Hippel-Lindau gene mutations in subjects highly exposed to TCE)	TCE used up to 1970s. Questionnaire by personal interview; secondary questions if TCE reported; employer records, hygienists from insurance companies.	Same plants as in Vamvakas study. Detailed info on TCE use, area descriptors—hot dip baths, small work areas, jobs, tasks—extensive cleaning; no controls.	Questionnaire, interview job histories and next of kin.	Scheme developed for cases by Vamvakas et al. (1998).	None in paper (Cherrie et al. [2001] extrapolated based on details in paper: peaks 400-600 ppm, long-term about 100 ppm).	Combination of symptom severity and frequency of exposures; ranked +, low; ++, medium; +++, high.

TABLE 3-10 *Continued*

Study	Qualitative Assessment	Information on Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Pesch et al. 2000a,b (incidence study)	Broad community study of five regions in Germany (included Arnsberg area).	Limited data on exposure setting; self-assessed exposure.	Questionnaire, interview job histories, agent use, and tasks.	Broad German and British JEMs, and local JEM based on job titles and tasks using self-reported information.	Expert judgment for who was exposed. No quantitative estimates.	Ever exposed; agent index based on duration, intensity, and probability of exposure.

Abbreviations: JEM, job exposure matrix; Perc, tetrachloroethylene; TCE, trichloroethylene.

assessments or through the use of job-exposure matrices, but the assessments were restricted to exposures to any solvents without identifying which were used (Harrington et al. 1989; Partanen et al. 1991; Dosemeci et al. 1999). In other studies, subjects reported whether they were exposed to specific agents (Sharpe et al. 1989; Mandel et al. 1995; Schlehofer et al. 1995; Vamvakas et al. 1998; Pesch et al. 2000a), and in some of those studies (e.g., Sharpe et al. 1989) rate ratios could have been overestimated because cases overreported exposure. In other studies, trichloroethylene was assessed specifically (Dosemeci et al. 1999; Parent et al. 2000; Pesch et al. 2000a; Brüning et al. 2003) and those studies may be more informative. In any risk assessment, the validity and reliability of the assessments of exposure have to be investigated closely.

Two small case-control studies conducted in the Arnsberg area of Germany found excess risks for exposure to trichloroethylene (Vamvakas et al. 1998; Brüning et al. 2003). The Vamvakas et al. (1998) study compared 58 renal cell cancer cases diagnosed between 1987 and 1992 with controls to provide an independent assessment of the Henschler et al. (1995) study. None of the cases in this study was included in the Henschler study. The odds ratios (OR) increased dramatically (OR = 1, 6.61, 11.92, and 11.42) with increasing exposures (categories of "no exposure," "+," "++," and "+++", respectively, based on exposure types, duration, and extent of prenarcotic symptoms). As summarized in Table 3-11, there were large differences in the severity of symptoms and duration of exposure by the amount of exposure. There were also large differences in the source of exposure, with the use of hot dip tanks (major sources of trichloroethylene vapors) predominating in the highest category and largely rag-and-bucket cleaning (limited local sources of trichloroethylene vapors) in the lowest category, which are consistent with large differences in the intensity of exposure. Thus, there is a high degree of consistency between symptom severity and reports of exposures.

TABLE 3-11 Prenarcotic Symptoms and Exposure Duration and Intensity Associated with Rated Exposure Levels in the Vamvakas et al. (1999) Study

	Rated Exposure Level		
Descriptor	+	++	+++
Prenarcotic symptoms (number, # category[a])	1, #1 3, #0	4, #3 9, #2	8, #3 2, #2
Frequency (number, daily or times per week)	1, daily 3, none	4, daily 4, 2 times per wk 5, 1 time per wk	5, daily 1, 3 times per wk 4, 2 times per wk
Exposure types	— — 3 rag and bucket 1 polishing	2 hot clean, 9 cold dip tanks, 1 rag and bucket, 1 dry clean	7 hot dip tanks; 1 welding on tanks with residues 2 cold dip tanks
Total duration (range)	Mean, 1,850 hr (1,100-2,500 hr)	Mean 4,141 hr (650-9,800 hr)	Mean, 28,800 hr (2,300-78,000 hr)

[a]Symptom grades were as follows: #0, none; #1, light symptoms (light dizziness, modest headaches); #2, moderate symptoms (light daze, clear dizziness, headaches); #3, severe symptoms (daze vertigo, severe headaches, and nausea, which did not permit the subject to remain exposed).

Another case-control study by Brüning et al. (2003) extended the period of observation from 1992 to 2000 and used the same rating scheme for exposure to trichloroethylene but used an independent set of cases and controls drawn from a wider geographic area. The validity of the data gathered by questions about neurological symptoms, which were asked of subjects in the Vamvakas et al. study, is a concern because legal proceedings were in progress to compensate workers for damage to their health. Two important considerations suggest that the workers' reports are valid. First, the Vamvakas et al. scale did not rely only on neurological symptoms but also included an assessment of duration and exposure intensity associated with the particular activities. Second, there are a variety of sensory cues that taken together can distinguish low and high vapor concentrations in addition to neurological symptoms. For example, at low exposures the vapors are colorless, nonirritating, and not pungent, but with high concentrations (greater than several hundred ppm) there are a variety of sensory cues: the vapors are irritating, have a strong odor, and were found to produce reduced performance and central nervous system symptoms in human volunteers during experiments in the 1960s (Stopps and McLaughlin 1967; also see Chapter 6). Thus, the workers' neurological symptoms were associated with other less subtle sensory responses, and they were only one dimension of the exposure evaluation. More importantly, overreporting would introduce misclassification, which would reduce the association between the symptoms and exposure.

The study by Vamvakas et al. (1998) has also been criticized in the literature; the essential observations are as follows:

1. Omission of cases from the Henschler et al. (1995) study (noted Mandel and Kelsh 2001). The effect of including these cases would lead to even higher estimates of risk.
2. Including only cases who worked in small industries and not applying the same criteria to controls (noted by Mandel and Kelsh 2001), which might lead to an underestimate of exposure among the control subjects and, thus, the odds ratios may have been overestimated. The committee shares the concern about this type of selection bias.
3. Cases were selected from one hospital but controls were selected from other hospitals in the area (noted by Green and Lash 1999; Mandel and Kelsh 2001). The committee is sanguine with regard to the selection of controls from other hospitals as it accepts the argument that these hospitals specialized in the type of care that they provided.
4. Prevalent cases (1987-1992) and controls (residual noncases) were selected in 1993 and interviews were conducted in 1993 (noted by Green and Lash 1999). McLaughlin and Blot (1997) suggested that survival in this period of time was 50% to 60%. Thus, some cases might have died in the interim before they could be interviewed and would have been excluded. This could have led to an inaccurate estimate of the exposure distribution. On the other hand, the control subjects who were enrolled when the interviews were conducted might not represent the true exposure distribution of the target population through time. In particular, exposures among the controls could have been underestimated if exposure diminished with time and if the selection of controls did not fully represent the actual distribution in the past (e.g., through changes in the population (immigration or emigration). Although this is a conjecture and the effects are difficult to predict, a sound design would have attempted to minimize such distortions. Thus, the committee is concerned about the possibility of a selection bias in this study and about the quality of the data obtained from subjects diagnosed in the past, especially if self-reported exposures were partially the basis of the assessments of exposure to trichloroethylene.

5. Interviewers (physicians) were aware of subjects' case status and surrogate respondents were used for deceased cases (but not for controls) (noted by Mandel and Kelsh 2001). One might expect that exposure of case subjects could have been overestimated because physicians more aggressively sought symptoms and exposure reports, although this was not possible for the deceased cases. It is unclear what overall effect this would have on the findings, although an analysis excluding the deceased cases would be useful.

6. Although the concentrations of exposure are unknown, the committee's analysis of the data in the Vamvakas et al. (1998) study, presented in Table 3-12 (see Appendix D for more detailed analysis of the this study), makes it clear that the severity of symptoms and the severity and duration of exposures were all substantial and consistent for the cases, and the controls as a group had fewer symptoms and lower exposures. The committee disagrees with the conclusions of some critics (Green and Lash 1999; Cherrie et al. 2001; Mandel and Kelsh 2001) that it was unclear how exposure to trichloroethylene was assessed. Table 3-12, prepared from the data of Vamvakas et al. (1998), clearly shows that graded differences on several scales are consistent with the ratings. Thus, a clear ordinal scale is present. However, the precise magnitude of exposures associated with these ratings is difficult to assess. Cherrie et al. (2001) separately estimated the exposure intensities with a suitable engineering model, which estimated peak exposures in the range of 500 ppm and averages about 100 ppm. The committee agrees with this assessment (see Appendix D). These exposures were consistent with the symptom reports in laboratory studies (Stopps and McLaughlin 1967).

TABLE 3-12 Trichloroethylene Exposure Summary for the Arnsberg Area Studies

Study	Peak Exposures	Long-term Exposures	Notes
Henschler et al. 1995	>2,000 ppm, machine cleaning with neurological symptoms; about 100 ppm continuous cold cleaning.	100 ppm 100 ppm	Cherrie et al. (2001) estimates
Vamvakas et al. 1998	400-600 ppm hot cleaning with neurological symptoms.	100 ppm	Cherrie et al. (2001) estimates
Bruning et al. 2003	400-600 ppm hot cleaning, with neurological symptoms.	100 ppm	

7. The control subjects were younger than the cases, implying a different potential for exposure to trichloroethylene (noted by Green and Lash 1999); therefore, risks could have been overestimated. If amounts of exposure have decreased and workers entered the workforce at about the same age across calendar periods, this could lead to an underestimate of exposure among controls, thereby leading to overstated risk ratios. However, responding to that criticism, the authors noted that there were no changes in exposure before 1986, when the allowable exposure was regulated. Given that the small enterprises have the highest exposures (Raaschou-Nielsen et al. 2002) and are usually the last to respond to regulations because of their limited resources, large employers are the initial focus of regulator activity. Further, the comment that younger workers would have lower exposures is not generally true because apprentices usually do the least skilled, dirtiest jobs; in the United States and Europe, younger workers have the highest exposures.

8. The authors did not find the two main accepted risk factors for renal cancer (smoking and obesity) (Mandel and Kelsh 2001). The main risk factors appear to be weakly associated with renal cancer, so not identifying these associations could be due to chance, lack of statistical power, or possibly to homogeneity of the population.

The study by Brüning et al. (2003) was carried out in a broader region of southern Germany, which included the Arnsberg region, by the same team of investigators but covered the calendar period 1992-2000 and a different set of cases and controls. Again, prevalent cases of renal cell carcinoma were identified in 1992-2000 and interviews were conducted in 1999-2000. Controls were identified and interviewed in 1999-2000 and were recruited from noncancer patients having surgery and from a local department of geriatrics. The geriatric department was used to enroll controls for patients who were older. Exposure was assessed on the basis of occupational history and self-reports of exposure to trichloroethylene and tetrachloroethylene, reports of prenarcotic symptoms for peak exposures using the same scheme as that in the Vamvakas et al. study, and the job exposure matrix of Pannett and the CAREX system to infer exposures. The committee judges that exposure range was likely similar to that in the Vamvakas et al. study, although they were drawing cases from a wider base population, which had a lower prevalence of exposures. For "ever exposed" to trichloroethylene, the investigators observed an OR of 2.47 and almost a 6-fold increase in risk among subjects who had daily occurrences of narcotic symptoms.

Some criticisms of this study are similar to those of the Vamvakas et al. study: (1) use of prevalent cases and residual noncases; (2) questions about the specific secondary study base that was used (surgery, geriatric clinics) and how representative it was of the target population; (3) whether interviewers were blinded and whether any of the authors were interviewers; and (4) whether surrogate respondents were interviewed as controls for deceased cases. In addition, although subjects were matched, there were noticeable differences in age (median age of cases, 68 years; median age of controls, 66 years).

The study by Vamvakas et al. exhibited a very large estimated OR of 10.8. The committee has concerns that the true exposure distribution of the target population was underestimated in the enrolled control series (criticisms 2, 4, 5, and 7 described above). Given the very large estimates of risk, a sensitivity analysis is warranted if these data are to be used in a risk assessment. The follow-up case-control study of Brüning et al. showed an OR of 2.47 for the same type of self-reported exposure to trichloroethylene but a broader more heterogeneous base population; the OR for jobs involved in metal degreasing that had potential exposure to trichloroethylene and tetrachloroethylene was 5.57. As the committee evaluated that the assessment of exposure in this study was similar to that of Vamvakas et al., this lower odds ratio might indicate bias in the Vamvakas et al. study or statistical variation between studies because the Brüning et al. study included a broader base population than that of the Vamvakas et al. and the Henschler et al. (1995) studies, which could have entailed a greater extent of misclassification of exposures. Despite these issues, the committee was impressed that three studies of the Arnsberg region of Germany, with very high apparent exposures and different base populations, showed a significant elevation of risk.

If there is doubt about the validity of a study, then the risk assessment can be conducted by including and excluding that study and determining the sensitivity of the findings. With regard to the study by Henschler et al., which shows much higher risks than the others, sensitivity analyses are warranted and their need can be argued by analogy to the standard

practice in epidemiology of assessing the effects of outliers; in risk assessment, this would be equivalent to the assessment of heterogeneity, except that the issue of a biased variance in the Henschler et al. study needs to be addressed.

Genetic Mutations and Kidney Cancer

Bruning et al. (1997a) described a possible somatic mutation in the Von Hippel-Lindau (*VHL*) tumor suppressor gene in the etiology of renal cell carcinoma arising from exposure to trichloroethylene. Brauch and coworkers have reported additional studies (Brauch et al. 1999; 2004). Brauch et al. (1999) compared somatic mutations among 44 cases with documented exposure to trichloroethylene in metal-processing plants in the Arnsberg region of Germany with 107 cases who had no such occupational exposure. The results of the study are summarized in Table 3-13. In addition, mutations at nucleotide 454 were found in 7 of the 17 high-exposure subjects and six of the 24 medium-exposure subjects, but no mutations were found in the three low-exposure patients or in 107 unexposed subjects.

Few details were presented about how subjects were selected, although they may have been recruited from the living subjects of the Vamvakas et al. (1998) case group. Whether there was blinding of exposure status in the assessments of somatic mutations was not stated.

In a second study, Brauch et al. (2004) reanalyzed cases from the Vamvakas et al. (1998) study for mutations in the *VHL* somatic gene. Thirty-eight of the original 58 patients with renal cell carcinoma were analyzed, and the authors used the original Vamvakas et al. exposure classification. Of the 17 exposed cases 15 had mutations and among the 21 unexposed cases 2 were found to have mutations (OR = 71.3). Because it was unclear to the committee why only this subset of cases was analyzed, a simple sensitivity analysis was conducted in which it was assumed that all 20 cases who were excluded were exposed but did not have any mutations. This analysis, which assumes extreme selection bias, still led to an OR of 6.5. An advantage of this "case-only" analysis is that it does not require use of the control series (analogous to a case-only study in gene-environment interactions); it was unclear whether there was blinding of exposure status when the molecular analyses were conducted.

TABLE 3-13 Number of Exposed and Unexposed Patients with *VHL* Gene Mutations

	VHL Mutational Status			
Trichloroethylene Exposure[a]	None	1	≥2	Total
High, +++	2	4	11	17
Medium, ++	6	15	3	24
Low, +	3	0	0	3
No documented occupational exposure[b]	31	42	0	73

[a]At the beginning of the discussion of the Brauch et al. (1999) paper it is noted that "The present study relies on the standardization of TRI [trichloroethylene] exposure levels of the RCC patients (10) …," where reference 10 is the Vamvakas et al. (1998) study; many of the coauthors are the same for both papers. Also in the paper by Brauch et al. (2004), p. 303, Table 1, a footnote notes that exposure data came from Vamvakas et al. (1998) subjects, and the case numbers of both Brauch et al. (1999, 2004) studies overlap. This indicates that the cases and their exposure assignments were obtained from the Vamvakas et al. (1998) study.

[b]The controls were drawn from other parts of Germany (populations assumed to be without the high prevalence of exposure to trichloroethylene) and evaluated by the same interview and questionnaire protocols.

Source: Adapated from Brauch et al. 1999.

Role of Metabolism in Trichloroethylene-Induced Renal Tumors

Extensive studies of trichloroethylene metabolism, coupled to its potential mechanism of action in nephrocarcinogenicity, have been reported (reviewed by Bruning and Bolt 2000). Trichloroethylene induces renal toxicity and renal tumors in rats (Maltoni et al. 1988; NTP 1988, 1990; EPA 2001). The nephrocarcinogenic effects of trichloroethylene are more pronounced in male rats, compared with female rats and were absent in male and female mice.[2] Studies of trichloroethylene metabolism in rodents and humans support a role for bioactivation in the development of nephrotoxicity and nephrocarcinogenicity after exposure to trichloroethylene (Lash et al. 1995, 2001a,b, 2002, 2003; Lash 2004). Trichloroethylene is metabolized by two competing pathways: oxidation by CYP450 and conjugation with glutathione (discussed earlier in this chapter; see Figure 3-1). Glutathione conjugation of trichloroethylene results in formation of *S*-(dichlorovinyl)glutathione, which is metabolized by enzymes of the mercapturic acid pathway (γ-glutamyl transpeptidase, aminopeptidase) to *S*-(1,2-dichlorovinyl)-L-cysteine, which is then metabolized by cysteine conjugate β-lyases, leading to the formation of electrophilic chlorothioketenes and sulfoxides. Concentrations of trichloroethylene in renal cortical homogenates have been reported to be generally two- to three-fold higher than in liver homogenates, and both oxidative and glutathione conjugation products were found in the liver and kidneys (Lash et al. 2006). These results are consistent with in vitro studies showing metabolism by kidney tissue. Males had substantially higher urinary excretion of *S*-(1,2-dichlorovinyl)-L-cysteine, suggesting greater metabolism by the glutathione pathway. It should be noted that results were reported for only three animals per time point and interpretation of the data is complicated by anomalous dose-concentration time profiles for trichloroethylene and its metabolites. The nephrotoxicity and nephrocarcinogenicity of trichloroethylene have been linked to the formation of *S*-(1,2-dichlorovinyl)-L-cysteine derivatives.

S-(1,2-Dichlorovinyl)-L-cysteine and its mercapturic acid metabolite *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine have been identified in the urine of humans exposed to trichloroethylene, providing evidence for the glutathione-dependent bioactivation of trichloroethylene in humans. Metabolism of trichloroethylene via the mercapturic acid metabolic pathway is consistent with the fact that the male rat is a sensitive species, because reduced glutathione (GSH) conjugation, γ-glutamyl transpeptidase, and cysteine conjugate β-lyase activity are all significantly higher in male than in female rats (Lash et al. 2002). Moreover, pharmacokinetic analysis of human volunteers after exposure to trichloroethylene (50 or 100 ppm) revealed that blood *S*-(dichlorovinyl)glutathione concentrations were 3.4-fold higher in males than in females, whereas clearance half-time values for systemic clearance of *S*-(dichlorovinyl)glutathione were similar in both genders (Lash et al. 1999). In the liver, metabolism of trichloroethylene via the mercapturic acid metabolic pathway is quantitatively less than via the CYP450-dependent metabolic pathway. However, the glutathione-dependent pathway becomes more pronounced when the oxidative metabolism of trichloroethylene is saturated in the case of high-dose exposure. Cummings and Lash (2000) demonstrated that human kidney tissue forms GSH conjugates with a K_m (0.58 mM) in the range of K_m values for oxidative metabolism by rodent microsomes (0.38 mM for mice, 0.07 and 0.48 mM for rats;

[2]Trichloroethylene is described in the literature as being carcinogenic in males only. The magnitude of effect is smaller in females and didn't reach statistical significance in the individual studies. EPA (2001) did an analysis of modified data. The results across strains were pooled and animals that died before any tumors were observed were removed from the analysis. With these modifications, the tumor effect in females was significant.

Table C-2). They reported minimal or nondetectable P450-mediated trichloroethylene metabolism in human kidney tissue.

Genotoxicity

Trichloroethylene causes a significant increase in the incidence of renal tumors in rats when administered orally and a marginal incidence of renal tumors when administered via inhalation; on the basis of limited evidence for carcinogenicity in humans and sufficient evidence for carcinogenicity in experimental animals, the International Agency for Research on Cancer (IARC 1995) classified trichloroethylene as a probable carcinogen in humans (group 2A). Moore and Harrington-Brock (2000) reviewed the genotoxicity of trichloroethylene and its glutathione-derived metabolites, and Bruning and Bolt (2000) reviewed the results of genotoxicity tests and concluded that trichloroethylene is, at most, a weak genotoxicant but noted that *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-L-cysteine have genotoxic effects including mutagenicity in the Ames test, unscheduled DNA synthesis, and formation of adducts in vitro with adenine, cytosine, and guanine. In the preliminary screening phase, the standard battery of genotoxicity tests might be unable to identify tissue-specific carcinogens, if the test system lacks the enzymes needed to form the toxic metabolite, and certainly does not provide any information on the possible species specificity of the test compound (Brambilla and Martelli 2004; Moore and Harrington-Brock 2000). Recently, Robbiano et al. (2004) applied both in vitro and in vivo assays to measure genotoxicity to kidneys of rodents and repeated the assays in primary cultures of human kidney cells. Six chemicals known to induce kidney tumors in rats, including trichloroethylene, were examined for their ability to induce DNA fragmentation and the formation of micronuclei in primary cultures of rat and human kidney cells and in kidneys of intact rats. Each chemical was tested at three to six concentrations (four 2-fold dilutions for trichloroethylene); the highest concentration tested produced a less than 30% reduction in survival. Significant dose-dependent increases in the frequency of DNA single-strand breaks and alkali-labile sites (as measured by the Comet assay) and in micronuclei frequency were obtained in primary kidney cells from male rats and from humans of both genders, with subtoxic concentrations of trichloroethylene. Among the six test compounds (benzofuran, bromodichloromethane, captafol, nitrobenzene, ochratoxin A, and trichloroethylene), trichloroethylene and bromodichloromethane exhibited the lowest DNA-damaging and micronuclei-inducing potencies (with ochratoxin A exhibiting the highest) in rats and humans. In agreement with these findings, statistically significant increases in the average frequency of both DNA breaks and micronucleated cells were observed in the kidneys of rats given a single oral dose (half the lethal dose to 50% of rats) of the six test compounds. For all these effects, the magnitude of the response was among the greatest for trichloroethylene. The results of this study also showed that the six rat kidney carcinogens produced genotoxic effects in primary cultures of human kidney cells that were quantitatively and qualitatively similar to those observed in primary cultures of rat kidney cells. Taken together, these findings provide evidence that trichloroethylene is genotoxic in short-term genotoxicity assays in kidney cells isolated from rats and human donors.

However, the authors noted limitations in the experimental design that limit interpretation and the significance of the above studies (Robbiano et al. 2004). These limitations include (1) examination of trichloroethylene on cells from only three donors, (2) considerable variation in

the frequency of DNA lesions induced in the cells, and (3) the possibility that kidney cells derived from kidney cancer patients could be more sensitive to DNA-damaging activity due to a more marked expression of enzymes involved in the metabolic activation of kidney procarcinogens and suppression of DNA repair processes. Therefore, the results of the genotoxicity studies must be considered solely as indicating that trichloroethylene might be genotoxic to the human kidney; the authors suggest that the designation of "inadequate evidence for carcinogenicity to humans" might not be tenable in the absence of sufficiently powered and carefully controlled epidemiologic studies.

Mode of Action

Role of von Hippel-Lindau Tumor Suppressor Gene

Most of the studies thus far reviewed on the subject of the renal carcinogenicity of trichloroethylene rely on either epidemiologic approaches or on studies of trichloroethylene metabolism and toxicity. The development of DNA technology and the discovery of tumor suppressor genes opened up a new route of investigation on the potential carcinogenic effects of trichloroethylene. Mutation or inactivation of the *p53* tumor suppressor gene is a common genetic alteration in human cancers. However, the *p53* gene is not a target in human and rat renal cell carcinoma (Reiter et al. 1993; Nishiyama et al. 1995). Inactivation of the *VHL* tumor suppressor gene in humans is responsible for the hereditary *VHL* cancer syndrome, predisposing affected individuals to a variety of tumors in specific target organs. More than 80% of sporadic renal cell carcinoma, but not papillary renal cell carcinoma, is associated with inactivation of the *VHL* gene (Gnarra et al. 1994). The *VHL* gene is only infrequently involved in extrarenal neoplasms, despite the broad range of *VHL* mRNA expression (including brain, adrenal, prostate, and lung), suggesting that its function as a tumor suppressor gene is specific for kidney epithelial cells (Walker 1998). The protein product of the *VHL* gene appears to regulate cell cycle arrest (transition from G_1 to G_0) by stabilizing the cyclin-dependent kinase inhibitor p27 (Soucek et al. 1998). Although the *VHL* gene, which is commonly mutated in human renal cell carcinoma, does not appear to be involved in rat renal cell carcinoma (Walker et al. 1996), it shares a common downstream effector (p27 that controls cell cycle progression) with the *TSC2* gene, a genetic target of renal cell carcinoma development in the rat. Because *VHL* is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis, future animal studies should use models in which target genes share common downstream signaling pathways with *VHL*.

One paper has linked the *VHL* gene to chemical-induced carcinogenesis. Shiao et al. (1998) demonstrated *VHL* gene somatic mutations in *N*-nitrosodimethylamine-induced rat kidney cancers that were of the clear cell type. The clear cell phenotype is rare in rat kidney cancers, but it was the only the clear cell cancers that showed *VHL* somatic mutation. This provided an additional link between *VHL* inactivation and clear cell kidney cancer.

Brauch et al. (1999, 2004) analyzed renal cancer cell tissues for mutations of the *VHL* gene and reported increased occurrence of mutations in patients exposed to high concentrations of trichloroethylene. In the first study (Brauch et al. 1999), subjects were identified from an occupational trichloroethylene exposure registry. They found multiple mutations in 42% of the exposed patients who experienced any mutation and 57% showed loss of heterozygosity. A hot

spot mutation of cytosine to thymine at nucleotide 454 (C454T) was found in 39% of samples that had a *VHL* mutation and was not found in renal cell cancers from nonexposed patients or in lymphocyte DNA from either exposed or nonexposed cases or controls. As discussed earlier, little information was given on how subjects were selected and whether there was blinding of exposure status during the DNA analysis.

In the second study, Brauch et al. (2004) investigated 38 renal cell carcinoma patients from a previous German case-control study performed by Vamvakas et al. (1998). Brauch et al. compared different renal cell carcinoma patient groups (trichloroethylene-exposed versus non trichloroethylene-exposed patients). The Vamvakas et al. study had described differences in renal cell carcinoma risks between trichloroethylene-exposed (n = 17) and nonexposed patients (n = 21). Brauch et al. (2004) extended the analysis by comparing age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the *VHL* tumor suppressor gene. Renal cell carcinoma did not differ with respect to histopathologic characteristics in both patient groups. Comparing results from trichloroethylene-exposed and nonexposed patients revealed clear differences with respect to (1) frequency of somatic *VHL* mutations, (2) incidence of C454T transition, and (3) incidence of multiple mutations. The latter is an indication that the effect of trichloroethylene is not limited to clonal expansion of cells mutated by some other agent. The C454T hot spot mutation was exclusively detected in tumors from trichloroethylene-exposed patients, as were multiple mutations. Also the incidence of *VHL* mutations in the trichloroethylene-exposed group was at least 2-fold higher than in the nonexposed group.

Brauch et al. were not able to analyze all the samples from the Vamvakas study, in part because samples were no longer available. Using the data described by Brauch et al. (2004) (*VHL* mutation found in 15 exposed and 2 nonexposed individuals, and *VHL* mutation not found in 2 exposed and 19 unexposed individuals), an OR of 71.3 is calculated. The most extreme example would be to assume that all 20 cases who were excluded were exposed but did not have mutations in *VHL* (*VHL* mutations were found in 15 exposed and 2 unexposed individuals and *VHL* was not found in 22 exposed and 18 unexposed individuals), which leads to an OR of 6.5, which remains significant.

Collectively, the data support the concept of a genotoxic effect of trichloroethylene leading to *VHL* gene damage and subsequent occurrence of renal cell carcinoma in highly exposed subjects. All the evidence, taken together, provides a consistent and plausible mechanism for a causal relationship and is strongly supportive of trichloroethylene being a human carcinogen after long-term exposure to high doses, such as occupational exposures described in both studies conducted in Germany (Henschler et al. 1995; Vamvakas et al. 1998).

The *VHL* gene is commonly altered in kidney tumors, especially those with the clear cell phenotypes. The alterations include loss of the entire or a large part of the gene (>90%) and small base changes (30% to 60%), including insertions, deletions, and point mutations (Shiao 2004). These changes can lead to reduction of protein expression, protein truncation, and incorrect amino acids incorporated into the protein (also called missense mutation). Consequently, wild-type constitutive functions of *VHL* are inactivated, with the subsequent potential to initiate and to promote tumor development. However, different mutations might have distinct tumorigenic potentials. Frequent and diverse *VHL* mutations in sporadic renal cell carcinoma provide a sizable mutation spectrum that has been used to correlate with environmental exposures. The rationale of using genetic signature as a marker of environmental exposure has been strengthened by in vitro and in vivo studies. Correlating of specific mutations

within the *VHL* gene with certain environmental exposures could lend support to the potential mutagenicity of an agent. Identification of DNA damage unique to exposure is necessary to provide strong evidence for the mutagenic potential of an environmental agent. Many types of DNA damage have been shown to induce unique signatures of gene mutations (see Table 3-14).

A worldwide mutation database compiling *VHL* mutations in sporadic renal cell carcinoma showed that missense mutations compose about 29% of all mutations; a large majority of base changes (71%) are nonmissense, including insertions, deletions, and frameshift alterations (see Table 3-15). When bases were determined, G:C to A:T, A:T to G:C, and A:T to C:G composed 48% of the changes. Similar mutation spectra have been obtained from cells and animals treated with alkylating agents, such as nitrosamines found in tobacco smoke and potent

TABLE 3-14 Mutation Spectra Indicative of Environmental Exposures and DNA Damage

Base Change	Possible Causes
Transition	
G:C to A:T	Deamination of 5-methyl-C or C; alkylation of G at O^6 position
A:T to G:C	Deamination of A; alkylation of T at O^2 or O^4 position
Transversion	
G:C to T:A	Mispairing of A with 8-OH-G or with apurinic G
A:T to T:A	Mispairing of A with apurinic A site
A:T to C:G	Misincorporation of 8-OH-G; error-prone repair of O^2- or O^4-alkyl T
G:C to C:G	Mispairing of G with oxidatively damaged G

Source: Shiao 2004. Reprinted with permission; copyright 2004, National Cancer Institute at Frederick.

TABLE 3-15 *VHL* Mutations in Sporadic Renal Cell Carcinomas

	Bruning et al. 1997a	Brauch et al. 1999		Brauch et al. 2004		UMD[a]
	Trichloroethylene Exposure					
	Yes	Yes	No	Yes	No	Unknown
Number of patients	23	44	73	17	21	
Patients with mutations	23 (100%)	33 (75%)	42 (58%)	14 (82%)	2 (10%)	
Number of mutation	23[b]	50	42	24	2	222
Missense	1	27 (54%)	NA	17 (71%)	2 (100%)	64 (29%)
Nonmissense	3	23 (46%)	NA	7 (29%)	0 (0%)	158 (71%)
G:C to A:T	1	21 (78%)	NA	12 (71%)	1 (50%)	21 (25%)
C to T at 454		(13)	(0/107)	(9)	(0)	(0)
G:C to T:A		0	NA	0	0	19 (22%)
G:C to C:G		5 (19%)	NA	4 (24%)	0	16 (19%)
A:T to T:A		1 (4%)	NA	1 (6%)	0	9 (11%)
A:T to G:C		0	NA	0	1(50%)	14 (16%)
A:T to C:G		0	NA	0	0	6 (7%)

Abbreviation: NA, not applicable.
[a]Universal Mutation Database (Beroud et al. 2000).
[b]By single strand conformation polymorphism (4 sequences confirmed).

human and animal renal carcinogens. The involvement of alkylating agents in the causation of renal cell carcinoma is further supported by the isolation of O^6-methylguanine and other alkylated DNA-damaged bases. However, mutation spectra after exposure to trichloroethylene or analog compounds, in cells and animals, have not been consistent. Nonetheless, increases in GC to AT and GC to TA mutations have been observed in bacteria. Muller et al. (1998) identified cytosine adducts from haloketene and halothioketene products of trichloroethylene; these are structurally similar to hydroxylamine cytosine adducts that result in C to T mutations (Budowsky 1976). Increases in *VHL* missense mutations, predominant in G:C to A:T base changes, and a hot spot of mutation at nucleotide 454, correlated with trichloroethylene exposure (Brauch et al. 1999). The three reports of trichloroethylene exposure from the same group suggest that trichloroethylene increases *VHL* mutations and generates a unique genetic signature of trichloroethylene exposure, which leads to the development of renal cell carcinoma. Although the findings linking trichloroethylene to renal cancer are of great consequence and relevance, further confirmation of mutagenicity and carcinogenicity at the molecular level is required to confirm the initial observations. As discussed earlier, consensus for the mutagenicity of trichloroethylene in mammalian cells remains to be established. If the mutation spectra in bacteria are considered, one would expect to see increases of both G:C to A:T and G:C to T:A mutations in trichloroethylene-exposed humans. However, a disproportionate number of G:C to A:T *VHL* mutations were reported (Brauch et al. 1999). Because alkylating agents, present in patients exposed to tobacco smoke, diuretic treatment for hypertension, and long-term dialysis for end-stage renal failure, also induce the same G:C to A:T base changes, analysis of prior trichloroethylene studies need to adjust for these risk factors. The temporal relationship between various mutations in the *VHL* gene and renal tumor progression needs to be examined more critically to unequivocally evaluate the cause-and-effect relationships. The mutagenicity of trichloroethylene should also be validated in additional cohorts. Further, the tumorigenic potentials of various *VHL* mutations need to be integrated, because mutated bases need not always be carcinogenic.

It remains debatable whether alterations in *VHL* alone are sufficient to trigger tumorigenic processes in the kidney, especially since experiments failed to detect any tumors in *VHL* knockout mice (Gnarra et al. 1997; Haase et al. 2001). Studies attempting to link the *VHL* gene to kidney tumor development are continuing in a variety of experimental models (Shiao et al. 1997, 1998; Walker 1998). However, there does not appear to be an experimental animal model with which to investigate the effects of trichloroethylene-induced mutations in the *VHL* gene and kidney tumor development.

Role of Nephrotoxicity in Trichloroethylene Renal Cancer

In animal studies, renal cancer occurs at high doses and is preceded by nephrotoxicity affecting the proximal tubule (NTP 1988, 1990). This has led to the proposal that nephrotoxicity is a prerequisite for the development of renal tumors and that exposures below nephrotoxic concentrations pose no risk of cancer. That is, there is a threshold exposure below which nephrotoxicity, and therefore renal cancer, will not occur (Bruning and Bolt 2000; Harth et al. 2005). In this scenario, nephrotoxicity, and subsequent cell division repairing that damage, functions as a promoter, allowing the expression of mutations (either spontaneous or induced by exposure to other agents, such as smoking and diuretics) within the renal cortex. Alternatively,

trichloroethylene is a complete carcinogen, with nephrotoxicity as the promoter for cells initiated by a trichloroethylene metabolite. There is evidence that trichloroethylene is genotoxic to human cells (Robbiano et al. 2004).

Nephrotoxicity is almost certainly secondary to formation of a toxic metabolite, and species differences in the extent of formation of that toxic metabolite could render humans less likely to develop nephrotoxicity and therefore cancer. The CYP2E1 and -3A5 isoforms that metabolize trichloroethylene have polymorphisms within national populations, resulting in considerable interindividual differences of enzyme expression. On a practical level, the population diversity in bioactivation and detoxification abilities could effectively obscure any threshold.

Investigations of nephrotoxicity in human populations have been pursued and the results show that highly exposed workers experience a tubular type of proteinuria, evidence of damage to the proximal tubule (Bruning et al. 1999a,b; Bolt et al. 2004). What is not clear is the magnitude of exposure needed to produce kidney damage. The fact that proteinuria was found in workers exposed to trichloroethylene concentrations that were not measured but were described as current occupational exposures (Green et al. 2004) is inconsistent with nephrotoxicity occurring only at high exposures that are not relevant to current occupational exposures.

FINDINGS

Although the committee was not charged with performing a risk assessment, it became clear from the epidemiologic evidence that there were sufficient data to make a recommendation about whether the findings of the mortality and incidence studies provided support for or against the hypothesis that exposure to trichloroethylene was associated with the induction of kidney cancer. There is strong evidence that exposure to high doses of trichloroethylene is associated with increased rates of kidney cancer. In particular, support for this conclusion derives from findings of increased risks in a cohort study (Henschler et al. 1995) and in case-control studies from the Arnsburg region of Germany (Vamvakas et al. 1998; Pesch et al. 2000a; Brüning et al. 2003). The committee notes that, as the designs of these case-control studies improved with time, increased risks were still observed. In addition, the finding of a mutation in the *VHL* somatic gene adds strength to these observations, although it would be useful if this finding were replicated in other settings. Of considerable interest was the finding of an increased risk among workers of a cardboard manufacturing plant in the United States (Sinks et al. 1992), who might have had exposures comparable to that in the study by Henschler et al. (1995). Other studies with appropriate power to detect risks from relatively low exposures also showed increased risks, notably the studies by Dosemeci et al. (1999), Raaschou-Nielsen et al. (2003), and Zhao et al. (2005).

Supporting this conclusion is the concordance between studies on humans and experimental animals for the site of tumors and occurrence of toxicity. In bioassay studies, rats developed tubular toxicity before tumors developed. Nephrotoxicity preceding cancer also appears likely in humans, although nephrotoxicity assessments in human studies were not made until after the development of renal cancer and were based on only one parameter.

The committee reviewed studies on two modes of toxicity proposed to be linked to cancer—accumulation of $\alpha_{2\mu}$-globulin and PPAR agonism. The committee concluded the evidence demonstrates these modes do not occur for trichloroethylene-induced renal cancer. The

committee also concluded that trichloroethylene causes an increase in the urinary excretion of formate but notes the disparities between formate-production and toxicity contradicts the conclusion that accumulation of formate is a mode of action for trichloroethylene nephrotoxicity.

Studies with experimental animals and human tissues support the conclusion that trichloroethylene, via one or more of its metabolites, is genotoxic. In animal studies, trichloroethylene appears to be a weak genotoxicant. The studies with human tissues used a small number of samples and, therefore, the committee notes this weakens the weight of evidence.

In the kidney, trichloroethylene can act as a complete carcinogen (at the stages of both tumor initiation and tumor promotion and progression) in a dose-dependent manner. Different types of kidney cancer can be triggered by different genes. After the discovery of the *VHL* tumor suppressor gene, it became recognized that homozygous inactivation of the *VHL* gene was linked to the occurrence of renal clear-cell carcinoma, the renal carcinoma preferentially induced by trichloroethylene. In exposed subjects, the genotoxic effect of trichloroethylene likely results from bioactivation pathways leading to renal *VHL* gene damage and renal cell carcinomas. The findings of experimental, mechanistic, and epidemiologic studies lead to the conclusion that trichloroethylene can be considered a potential human carcinogen.

RESEARCH RECOMMENDATIONS

- Because sulfoxide metabolites are more potent nephrotoxicants than their parent *S*-conjugates, more research is needed on the extent of formation of *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxides by human tissues (liver and kidney), the extent to which these reactions occur in vivo, the enzymes involved, and their interindividual variability, including the role of genetic polymorphisms. The toxicologic significance of trichloroethylene or *S*-(1,2-dichlorovinyl)-L-cysteine *S*-conjugate sulfoxidation products also should be evaluated.
- High frequencies of missense mutations in the *VHL* gene do not constitute a cause of renal cell carcinoma; the tumorigenic potential of missense mutations in the *VHL* gene should be determined. The potential of specific missense mutations in the *VHL* gene contributing to tumor initiation and progression should be determined.
- Although correlation of *VHL* mutations to trichloroethylene exposure and renal cell cancer are persuasive, the findings need to be validated in other populations and geographic areas. Because many risk factors for renal cell carcinoma generate mutation spectra similar to that of trichloroethylene, coexposure to trichloroethylene with other risk factors needs to be seriously considered and accounted for in future epidemiologic studies.
- Mechanistic studies should include field studies of populations exposed to trichloroethylene to assess the range of metabolic pathways used and relative amounts of metabolites from each pathway as a function of exposure intensity and enzymatic genotypes. This information will greatly help in the interpretation and extrapolation of information from rodents to humans.
- Additional studies of nephrotoxicity in workers exposed occupationally to trichloroethylene should be performed. It is important that actual exposures are measured and not estimated using biological markers that are subject to large interindividual differences.

- No analytic community studies were included in the committee's assessment of kidney cancer. Given the importance of contamination of water supplies by trichloroethylene, it is important that sufficiently robust studies (with sufficient statistical power and exposure assessments) be conducted in the general population where such exposures might be occurring.
- Any follow-up epidemiologic study must have a wide range of exposures, preferably to the range of the Vamvakas and Henschler studies to provide an anchor in that range where effects were seen. There may be opportunities for studies of populations in developing countries in Asia and Eastern Europe, where high exposures to trichloroethylene may not have been controlled. Strong, quantitative exposure assessments will be critical for these studies to be useful for resolving the remaining dose-response issues.

4

Liver Toxicity and Cancer

This chapter reviews information on the effects of trichloroethylene and its principal metabolites (trichloroacetic acid, dichloroacetic acid, and chloral hydrate) on the liver, particularly information generated since the U.S. Environmental Protection Agency (EPA) released its draft health risk assessment (EPA 2001b). Trichloroethylene metabolism occurs primarily in the liver and is critical to understanding its toxicity and carcinogenicity. Background information on trichloroethylene metabolism is provided in Appendix C. In this chapter, hepatotoxicity and liver cancer are discussed separately, although they are not necessarily independent end points. A review of current knowledge on the proposed modes of action for trichloroethylene-induced liver cancer (peroxisome proliferator-activated receptor agonism, genotoxicity and mutagenicity) and their relevance to humans is provided.

HEPATOTOXICITY

Animal Studies

It is well documented that trichloroethylene produces hepatotoxicity in experimental animals and humans (ATSDR 1997a; EPA 2001b). Table 4-1 provides the details of some recent studies, and selected findings are discussed below.

Rodents exposed to high doses of trichloroethylene or some of its metabolites develop hepatocellular necrosis. Different studies have localized the injury to midzonal, periportal, or centrilobular hepatocytes (Buben and O'Flaherty 1985; Soni et al. 1998, 1999; Lee et al. 2000). This lack of consistency in location of injury might reflect the routes of administration, doses, strain, or species of rodents used in the different studies. For example, Soni et al. (1999) conducted dose-response studies with trichloroethylene (250-2,500 mg/kg) to investigate the time course of liver injury and compensatory hepatocyte regeneration. Hepatocellular necrosis was evident after 24 hours at all doses. Injury was detected in midzonal areas of the liver lobule with no evidence of necrosis in hepatocytes adjacent to the central vein (centrilobular hepatocytes). This study also showed that the dose of trichloroethylene can influence the location of injury. At a trichloroethylene dose of 2,500 mg/kg, centrilobular injury was clearly

TABLE 4-1 Hepatotoxicity of Trichloroethylene and Metabolites in Animal Studies

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
Swiss-Cox mice (males)	0-3,200 mg/kg/day, TCE	6 wk	Gavage/corn oil	Increases in serum glutamic pyruvic transaminase evident only with the two highest doses; histologic findings: swollen hepatocytes and minimal evidence of necrosis.	Buben and O'Flaherty 1985
Sprague-Dawley rats (males)	250, 500,1,250, 2,500 mg/kg, TCE	Single dose	i.p./corn oil	Midzonal injury that spreads to centrilobular regions with the highest dose.	Soni et al. 1998, 1999
Sprague-Dawley rats (males)	16 and 64 mg/kg, TCE	Single dose	i.v. or via portal vein cannula/vegetable oil	Periportal necrosis with portal vein administration. No necrosis with i.v. administration. This is reflective of solvent-related injury of TCE to portal areas.	Lee et al. 2000
Autoimmune prone $MRL^{-/-}$ mice (female)	0, 0.1, 0.5, 2.5 mg/mL, TCE	4 and 32 wk	Drinking water	$CD4^+$ T-cell activation; mononuclear infiltration in portal regions consistent with autoimmune hepatitis; slight but significant increase of serum ALT.	Griffin et al. 2000a
Autoimmune prone $MRL^{-/-}$ mice (female)	0 or 2.5 mg/mL, TCE in presence of diallyl sulfide (CYP2E1 inhibitor)	4 wk	TCE in drinking water; diallyl sulfide via osmotic pump	Blockade of TCE protein adduct formation; reversal of $CD4^+$ T-cell-mediated autoimmunity by TCE.	Griffin et al. 2000b
Autoimmune prone $MRL^{-/-}$ mice (female)	0, 0.1, 0.9 mg/mL, TCA or chloral	4 wk	Drinking water	$CD4^+$ T-cell activation by these metabolites as shown for TCE.	Blossom et al. 2004
Wistar rats (male)	376 ppm TCE, 4 hr/day, 5 days/wk	8, 12, 24 wk	Inhalation	This dose (1/25 LC_{50}) resulted in hepatomegaly and fatty infiltration; fatty changes with marked necrosis were more evident at 12 and 24 wk. No elevation in serum liver transaminases or mortality was detected.	Kumar et al. 2001a

TABLE 4-1 *Continued*

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
B6C3F$_1$ mice (female)	25, 50, and 100 mg/kg chloral hydrate, 5 days/wk	3, 6, 12 mo, 2 yr	Gavage/in water	No evidence of dose-dependent elevation in serum liver transaminases, only AST elevated at the 50-mg/kg dose.	NTP 2002b
Swiss-Webster mice (male)	15, 30, 75 mg/kg, DCVC	Single dose	i.p./in water	Transient elevation in liver transaminases at the highest dose; no histologic evidence of liver injury.	Vaidya et al. 2003a
B6CF$_1$ mice (male)	1 g/L chloral hydrate; 0.5 g/L, DCA	104 wk	Drinking water	Increased liver weight and hepatocellular necrosis with both metabolites.	Daniel et al. 1992
B6C3F$_1$ mice (male)	0.5 or 5g/L, DCA	0, 5, 15, 20, 30 days	Drinking water	Dose- and time-dependent liver enlargement; morphologic evidence of focal necrosis and apoptotic bodies.	Carter et al. 1995
B6C3F$_1$ mice (male and female)	1 or 2 g/L, DCA or TCA	52 wk	Drinking water	Enlarged livers, significant glycogen accumulation, focal areas of necrosis seen with DCA. No focal necrosis with TCA, modest hyperthrophy and glycogen accumulation.	Bull et al. 1990
B6C3F$_1$ mice (male and female)	1 or 2 g/L, DCA or TCA	52 wk	Drinking water	Hepatocytes from DCA-treated mice contained large amounts of glycogen evenly distributed throughout the liver; less glycogen accumulation with TCA treatment, which was more prominent in periportal regions.	Bull et al. 1990

TABLE 4-1 *Continued*

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
B6C3F$_1$ mice (male)	0.1 to 3 g/L, DCA	1, 2 and 8 wk	Drinking water	Dose-dependent liver glycogen accumulation associated with decreased glycogen synthase activity. No effect in glycogen phosphorylase or glucose-6-phosphatase (enzymes involved glycogen metabolism).	Kato-Weinstein et al. 1998
B6C3F$_1$ mice (male)	0.1 to 2 g/L, DCA	2-10 wk	Drinking water	Significant reduction in serum insulin levels, insulin receptor expression, and protein kinase B. Increases in liver glycogen preceded these changes.	Lingohr et al. 2001
Fresh hepatocytes in culture from B6C3F$_1$ mice (male)	10-500 μM, DCA	16-hr incubation	N/A	Dose- and time-dependent glycogen accumulation. Presence or absence of insulin in culture media did not affect this DCA effect. However, glycogen accumulation is dependent on phosphatidylinositol 3-kinase activity.	Lingohr et al. 2002
B6C3F$_1$ mice (female)	3.2 g/L, DCA with or without L-methionine at 4 or 8 g/kg	8 and 44 wk	DCA in drinking water; L-methionine in diet	L-Methionine prevented liver DNA hypomethylation completely, while blocking only 25% of glycogen accumulation produced by DCA.	Pereira et al. 2004
Fisher 344 rats (male) and B6C3F$_1$ mice (female)	0, 1, 2 g/kg, dibromoacetic acid	2, 4, 7, 28 days	Drinking water	Dose- and time-dependent hypomethylation of liver DNA in both species. Significant increases in liver glycogen in both species, although longer exposure is required in rats for this effect.	Tao et al. 2004a

TABLE 4-1 *Continued*

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
Sprague-Dawley rats (males)	0.01, 0.1, 1, 5, and 10 mmol/kg, TCE	Daily dosing for 3 days	i.p./corn oil	Increases in total and some individual serum bile acids (TC was most sensitive). No elevation in transaminases (except for ALT at the highest dose) or morphologic evidence of injury. Separate inhalation studies showed similar elevation in serum TC levels.	Wang and Stacey 1990
Fresh hepatocytes in culture from Sprague-Dawley rats (males)	Cells were dosed by vapor phase in 25-mL flasks; exposure: 0, 0.5, 1, 2, 4 μL per flask, TCE	20-min exposure	N/A	Dose-dependent inhibition of bile acid (TC) uptake into hepatocytes. Time-dependent inhibition of bile acid accumulation. No significant intracellular enzyme or potassium leakage. No changes in bile acid efflux from hepatocytes with TCE exposure.	Bai and Stacey 1993
Fresh hepatocytes in culture from Sprague-Dawley rats (males)	Cells were dosed by vapor phase in 25-mL flasks; exposure: 0, 2, 5, 10 μL per flask (concentration range: 230-1,000 μM), 1,1,1-trichloroethane	20-min exposure	N/A	No ALT, lactate dehydrogenase or potassium leakage at any dose level. Concentration-dependent inhibition of TC, ouabain, and 2-aminoisobutyric acid uptake. Decrease ATP levels and activity of ATP-dependent membrane ATPases. No overt morphologic changes in TCE-exposed hepatocytes.	Kukongviriyapan et al. 1990

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCA, dichloroacetic acid; DCVC, *S*-1,2-dichlorovinyl-L-cysteine; i.p., intraperitoneal; i.v., intravenous; LC_{50}, concentration lethal to 50% of test animals; N/A, not applicable; ppm, parts per million; TC, taurocholate; TCA, trichloroacetic acid; TCE, trichloroethylene.

evident after 24 hours. High doses of trichloroethylene administered into the portal vein caused periportal liver injury via a direct solvent action rather than a mechanism dependent on activation by the enzyme cytochrome P-450 (CYP-450) (Lee et al. 2000).

Reynolds and Moslen (1977) proposed that reactive intermediates of trichloroethylene generated by CYP-450 bind covalently to cellular components, resulting in cell necrosis. More recent evidence from mouse studies suggests that an autoimmune response might play a role in trichloroethylene-mediated liver disease (Griffin et al. 2000a). Administration of trichloroethylene at concentrations of 0 to 2.5 mg/mL in drinking water to autoimmune-prone $MRL^{+/+}$ mice for 34 wk resulted in an inflammatory response in the liver. Metabolic activation of trichloroethylene by CYP2E1 was demonstrated to be an obligatory step in the development of autoimmune hepatitis in the mice. The metabolites trichloroacetic acid and chloral hydrate also have the potential to induce autoimmunity in the same autoimmune-prone mice (Blossom et al. 2004).

Recent studies investigated the hepatotoxicity produced by trichloroethylene in rats exposed via inhalation at 376 parts per million for 8, 12, or 24 wk. Liver enlargement with necrotic cells and fatty infiltration was more prominent in rats in the 12- and 24-week treatment groups. The authors also detected elevated markers of lysosomal disruption. They recorded no mortality in any of the treatment groups (Kumar et al. 2001a).

Human Studies

Table 4-2 presents findings from human studies of hepatotoxicity. There is some evidence that occupational exposure to trichloroethylene results in several forms of non-cancer liver disease such as hepatic necrosis, fatty liver, and cirrhosis. It is well established that acute occupational exposure to trichloroethylene does not produce liver injury, whereas chronic exposure does. Case reports have linked occupational exposure to trichloroethylene with Stevens-Johnson syndrome (erythema multiforme major) of abrupt onset (Phoon et al. 1984). All these cases demonstrated liver involvement ranging from mild jaundice to fatal liver failure. Another case report documented that repeated exposure to trichloroethylene in the work setting resulted in chronic cirrhosis and portal hypertension (Thiele et al. 1982). The most recent report of trichloroethylene hepatotoxicity associated with occupational exposure comes from a watch manufacturing plant in Thailand, where two female workers developed generalized skin lesions, fever, and hepatitis. One case resulted in fatal hepatic damage 2 weeks after the onset of symptoms. Both workers cleaned watch metal straps by dipping them in containers that contained trichloroethylene (Pantucharoensri et al. 2004).

These case reports support data from animal studies indicating that an autoimmune response might be important in trichloroethylene-induced hepatitis. Genetic and environmental factors that influence xenobiotic metabolizing enzymes can favor the formation of trichloroethylene metabolites capable of triggering an immune response against the liver.

Contribution of Metabolites to Hepatotoxicity

Chloral hydrate, a metabolic intermediate of trichloroethylene, has been reported to contribute to the hepatotoxic potential of this solvent. In a 2-year National Toxicology Program

TABLE 4-2 Hepatotoxicity of Trichloroethylene and Metabolites in Human Studies

Subjects	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
Five case reports (males and females)	50-912 mg/m^3, TCE	3-5 wk	Inhalation of vapors in workplace	Stevens-Johnson syndrome (erythema multiforme), jaundice, hepatomegaly, and hepatic encephalopathy. Other solvents besides TCE might be involved.	Phoon et al. 1984
Two case reports (females)	15-45 ppm, TCE	4-5 wk	Inhalation of vapors in workplace	Stevens-Johnson syndrome, generalized skin eruptions, and hepatitis with no jaundice (case 1). Fulminant hepatitis (case 2).	Pantucharoensri et al. 2004
Cross-sectional study (148 workers) and a 2-yr follow-up study (13 workers)	Low, moderate, and high TCE exposure based on concentrations of total trichloro compounds detected in urine	Duration of employment: 0.1 to 36.6 yr; average: 7 yr	Ambient air; occupational	Increases in high density lipoprotein cholesterol in the absence of elevation in plasma liver transaminases, indicating that low level exposure to TCE affects cholesterol metabolism without causing hepatocellular necrosis. Alcohol intake is an influential factor in the cross-sectional study.	Nagaya et al. 1993
Human workers (21 men, 1 woman)	Regular exposures of less than 5 ppm TCE; peak exposures for 2 workers at over 250 ppm	Mean duration of employment for TCE exposed workers: 7 yr	Ambient air; occupational	Highly significant increases in individual and total serum bile acids in the exposed group (controlled for age and alcohol intake). No abnormalities in liver function tests. No relationship between plasma bile acid and cholesterol was detected.	Driscoll et al. 1992
Human workers	8.9 + 3.1 ppm TCE	Mean duration of exposure: 3.4 yr	Ambient air; occupational	Elevation in total serum bile acids and some individual bile acids; normal hepatobiliary function tests.	Neghab et al. 1997

Abbreviation: TCE, trichloroethylene.

(NTP 2002b) study, male B6C3F$_1$ mice given chloral hydrate by gavage at 25, 50, or 100 mg/kg showed no significant changes in three serum liver transaminases, except for a significant increase in aspartate aminotransferase activity in the 50-mg/kg group.

Lash et al. (1995) investigated the toxicity of trichloroethylene and its metabolites with freshly isolated rat hepatocytes in culture. The studies showed that exposure to only *S*-(1,2-dichlorovinyl)-L-cysteine resulted in hepatocellular damage. None of the other metabolites (CYP450 dependant or reduced glutathione dependent) or trichloroethylene produced hepatocellular injury. The metabolites tested included trichloroacetic acid, dichloroacetic acid, chloral hydrate, trichloroethanol, oxalic acid, and *S*-1,2-dichlorovinyl-L-glutathione. Despite this lack of cytotoxicity, trichloroethylene and its metabolites produced mitochondrial dysfunction. This in vitro study showed that *S*-1,2-dichlorovinyl-L-cysteine is the only trichloroethylene-derived compound cytotoxic to rat hepatocytes in culture.

In contrast, in vivo studies showed that *S*-1,2-dichlorovinyl-L-cysteine has a very low hepatotoxic potential. Acute toxicity studies investigating the nephrotoxicity of *S*-1,2-dichlorovinyl-L-cysteine in male Swiss-Webster mice showed transient elevations in serum liver transaminases 12 hours after administration of the highest dose tested (75 mg/kg). This dose resulted in significant lethality due to nephrotoxicity at later times. No liver histopathology was detected at any of the doses or time points examined (Vaidya et al. 2003a).

Other studies investigating the epigenetic mechanisms of dichloroacetic-acid-induced carcinogenesis revealed morphologic evidence of liver injury that includes loss of cell membrane integrity, focal areas of cell debris, and appearance of apoptotic bodies in B6C3F$_1$ mice undergoing short-term exposure to dichloroacetic acid at 0.5 or 5 g/L for up to 30 days (Carter et al. 1995). Hepatocellular necrosis was also detected in male and female Swiss-Webster mice receiving dichloroacetic acid in their drinking water at 300, 1,000, or 2,000 mg/L for up to 14 days. This was accompanied by a marked increase in liver weights. No such changes were seen with trichloroacetic acid under the same dosing regimen (Bull et al. 1990; Sanchez and Bull 1990). Exposure of male B6C3F$_1$ mice to dichloroacetic acid (0.5 g/L) or chloral hydrate (1 g/L) via drinking water resulted in hepatocellular necrosis after 104 wk of exposure (Daniel et al. 1992).

Changes in Liver Glycogen Status

Exposure to trichloroethylene produces effects in the liver other than hepatocellular injury. Treating mice with dichloroacetic acid results in marked dose-dependent accumulation of liver glycogen (Bull et al. 1990; Kato-Weinstein et al. 1998). This dose-response relationship parallels that for the development of hepatocellular carcinomas. Furthermore, patients with glycogen storage disorders have a greater propensity for developing liver tumors (Labrune et al. 1997). These observations have prompted investigators to study in depth the relationship between increased liver glycogen storage and carcinogenesis.

Studies have been carried out to assess the effect of dichloroacetic acid treatment on insulin secretion, insulin receptor expression, and activity and expression of protein kinases controlled by insulin receptor signaling due to the role of these gene products in glycogen synthesis and homeostasis (Lingohr et al. 2001). Mice treated with dichloroacetic acid in drinking water at 0.1 to 2.0 g/L for 2-10 weeks showed a significant reduction in expression of the insulin receptor in the liver. As early as 2 weeks after the initiation of dichloroacetic acid

treatment, insulin concentrations were significantly reduced. Dichloroacetic acid similarly reduced the expression of protein kinase B (an insulin-sensitive enzyme involved in glycogen homeostasis). Because dichloroacetic-acid-induced glycogen accumulation precedes down-regulation of the insulin receptor and insulin-dependent signaling pathways, these changes in gene expression for insulin and related genes are considered to be compensatory responses to changes in glycogen homeostasis.

Lingohr et al. (2002) investigated whether the changes in glycogen accumulation brought about by dichloroacetic acid were insulin dependent. Freshly isolated mouse hepatocytes exposed to dichloroacetic acid accumulated more glycogen than control hepatocytes. This response was dose dependent. Omitting insulin from the culture media did not prevent the enhanced accumulation of glycogen produced by dichloroacetic acid. By contrast, dichloroacetic-acid-induced glycogen deposition was fully blocked by inhibition of the enzyme phosphatidylinositol 3-kinase. Phosphatidylinositol 3-kinase participates in the signal transduction pathway leading to glycogen synthesis initiated by activation of the insulin receptor. This suggests that dichloroacetic-acid-induced glycogen accumulation involves a phosphatidylinositol 3-kinase-dependent pathway downstream of the insulin receptor (Lingohr et al. 2002). In addition to regulating glycogen synthesis, phosphatidylinositol 3-kinase has been implicated in the proliferative and anti-apoptotic effects of peroxisome proliferators (Mounho and Thrall 1999), which further establishes an association between abnormal liver glycogen status and the carcinogenic effect of trichloroethylene and its metabolites. Other halogenated solvents such as bromochloroacetate and dibromoacetate induce glycogen accumulation in the liver to a similar degree as dichloroacetic acid (Kato-Weinstein et al. 2001).

Another potential link between aberrant liver glycogen homeostasis and the carcinogenicity of trichloroethylene is provided by the effect of this chemical and its metabolites on DNA methylation status. Exposure to dichloroacetic acid results in hypomethylation of protooncogenes and other genes involved in cell growth, such as insulin-like growth factor II in mouse liver (Tao et al. 2000a, 2004b; Ge et al. 2001). This response has been causally linked to the development of hepatocellular adenomas and carcinomas induced by these chemicals.

Dibromoacetic acid, a related haloacetic acid, similarly induced hepatic DNA hypomethylation in female mice and male rats receiving 1 or 2 g/L in their drinking water for up to 28 days. Specifically, hypomethylation of c-*myc* and insulin-like growth factor II was detected along with increases in mRNA expression of both genes in mouse liver. Only c-*myc* mRNA expression was increased in rat liver. Glycogen accumulation and induction of markers of peroxisome proliferation were also observed in mice and rats receiving dibromoacetic acid (Tao et al. 2004a). These results further support the relationship between glycogen accumulation and liver tumors induced by trichloroethylene and its metabolites, making it more uncertain whether trichloroethylene-induced glycogen accumulation can be considered a noncancer liver effect or an early event in carcinogenesis. More recently, Pereira et al. (2004) showed that methionine treatment not only prevents the DNA hypomethylation induced by dichloroacetic acid and related solvents, but it also prevents liver tumor formation in mice. Interestingly, methionine did not prevent glycogen accumulation completely. Dichloroacetic-acid-induced glycogen storage was reduced by only 25% with methionine treatment. This new information suggests dissociation between glycogen accumulation and the carcinogenic effects of dichloroacetic acid. Clearly, more studies are needed to clarify the relationship between altered glycogen storage and liver cancer in response to trichloroethylene.

Elevation of Serum Bile Acids

Occupational exposure to trichloroethylene has been reported to increase serum bile acid and cholesterol concentrations. Chronic occupational exposure to low concentrations of trichloroethylene appears to alter cholesterol metabolism in the absence of noticeable hepatocellular damage, as evidenced by lack of increase in serum liver transaminases (Nagaya et al. 1993). Similarly, serum concentrations of total and individual bile acids were significantly elevated in a group of workers exposed to trichloroethylene (Driscoll et al. 1992; Neghab et al. 1997). Because no association was observed between elevated plasma bile acids and conventional markers of liver injury, it was concluded that this perturbation in bile acid homeostasis could be indicative of early changes in liver function independent of hepatocellular damage.

Similar alterations in bile acid status have been observed in experimental animals exposed to trichloroethylene and its metabolites. Exposure to trichloroethylene via inhalation or intraperitoneal injection resulted in elevation of serum bile acid concentrations at doses that did not produce changes in markers of liver function, such as serum liver transaminases and bilirubin concentrations (Wang and Stacey 1990). To determine whether this increase in serum bile acids after low exposure to trichloroethylene is indicative of early liver dysfunction, the mechanism(s) responsible for these changes was investigated by examining the effect of trichloroethylene on bile acid transport in freshly isolated rat hepatocytes in culture (Bai and Stacey 1993). The uptake of the bile acids taurocholic and cholic acids into rat hepatocytes was inhibited in a dose-dependent manner by trichloroethylene at concentrations up to 1.84 mM. These concentrations did not result in significant leakage of transaminases or intracellular potassium into the culture media. Trichloroethylene inhibition of bile acids uptake was determined to be noncompetitive. These results indicate that the increase in serum bile acids produced by trichloroethylene occurs through interference with uptake into hepatocytes. The same study also determined that trichloroethylene does not affect the efflux of bile acids from hepatocytes. These observations clearly show that alterations in transport processes for bile acids (primarily by inhibiting uptake) occur in the absence of pathologic evidence of liver dysfunction.

The fact that inhibition of bile acid uptake by trichloroethylene was determined to be noncompetitive strongly argues against direct competition between trichloroethylene and bile acids, such as taurocholic and cholic acids, for the main basolateral carrier in hepatocytes for bile acids, the sodium taurocholate transporter polypeptide. This effect on bile acids is not unique for trichloroethylene, because occupational exposure to a mixture of other organic solvents including toluene, xylene, acetone, *n*-butanol, and ethylacetate similarly increases serum bile acids (Franco et al. 1986). These results also argue against competition for common transport as it is very unlikely that the uptake of all these solvents into hepatocytes requires the same transport process. Because of their high lipid solubility, these solvents can readily partition into the plasma membrane of hepatocytes by diffusion, which does not require transport protein function.

A decrease in hepatic bile acid uptake by trichloroethylene can be the result of changes in or disruption of plasma membrane lipids and changes in membrane fluidity. Changes in the fluidity of the plasma membrane lipid bilayer are known to affect the function of uptake transporters and other transmembrane proteins. Again, this is supported not only by the noncompetitive nature of the inhibition of the uptake of bile acids by trichloroethylene but also by its reversibility with time. In contrast, the lack of changes in bile acid efflux in trichloroethylene-exposed rat hepatocytes does not support the idea that changes in membrane

fluidity contribute to overall reduction in plasma membrane transport protein function, because changes in membrane fluidity should also affect the function of plasma membrane efflux transporters.

Earlier studies also demonstrated that 1,1,1-trichloroethane, a solvent similar to trichloroethylene, decreases ATP concentrations and inhibits the activity of plasma membrane ATPases in cultured rat hepatocytes in a dose-dependent manner. The only ultrastructural alteration detected in 1,1,1-trichloroethane-exposed hepatocytes was loss of membrane microvilli that was not associated with cell death (Kukongviriyapan et al. 1990). This reduction in ATP concentration could lead to impairment of energy-dependent transport processes across the plasma membrane of hepatocytes, thus providing a possible mechanistic explanation for the reduction in bile acid uptake into the liver of experimental animals. On the other hand, the main efflux transporter for bile acids in hepatocytes, known as the bile-acid-exporting pump, is also an ATP-dependent carrier. A reduction in cellular ATP quantities by 1,1,1-trichloroethane should also affect the efflux of bile acids from hepatocytes via the bile-acid-exporting pump if a reduction in cellular ATP status is a critical factor in the abnormal handling of bile acids with 1,1,1-trichloroethane exposure. However, this is not the case. Additional studies are required to better define the mechanisms by which trichloroethylene affects the vectorial transport of bile acids across hepatocytes.

The interference of 1,1,1-trichloroethane with the influx of chemicals into hepatocytes is not limited to bile acids because 1,1,1-trichloroethane also inhibited the uptake of ouabain and 2-aminoisobutyric acid (Kukongviriyapan et al. 1990). These two compounds are known to enter hepatocytes via an energy-dependent transport-mediated process. It is worth noting that the transport of cadmium chloride and 3-*O*-methyl-D-glucose does not change. Furthermore, occupational exposure to a mixture of organic solvents including toluene, xylene, acetone, *n*-butylacetate, *n*-butanol, and ethylacetate also results in elevation of mean serum bile acid concentrations in the absence of changes in biochemical markers of liver injury (Franco et al. 1986). Exposure to toluene by itself produces the same response (Neghab and Stacey 1997).

LIVER CANCER

This section provides an overview of the evidence on liver cancer caused by trichloroethylene from animal and human studies. Tables detailing dose-response data from animal studies are included to provide a context for assessing risks from environmental exposures and to conduct physiologically based pharmacokinetic modeling. The potential modes of action of carcinogenesis for trichloroethylene and its metabolites are then discussed.

Animal Studies

Trichloroethylene

Trichloroethylene induction of hepatic tumors in rodents was extensively reviewed by Bull et al. (2002). Gavage administration of trichloroethylene in corn oil has been shown to produce hepatic cancer in B6C3F_1 mice (NCI 1976; NTP 1990). One study (NCI 1976) used technical grade trichloroethylene (1,000 and 2,000 mg/kg for males, 700 and 1,400 mg/kg for

females) that contained 0.09% epichlorohydrin, a known rodent carcinogen. In another study (NTP 1990), a single dose of epichlorohydrin-free trichloroethylene was administered by gavage in corn oil to male and female B6C3F$_1$ mice at 1,000 mg/kg/day. Significant increases in the incidence of hepatocellular cancer were found in the mice.

Both pure amine-based-stabilized trichloroethylene and technical grade trichloroethylene with stabilizers (epichlorohydrin and 1,2-expoxybutane) were tested at 1.8 or 2.4 g/kg in corn oil in Swiss ICR/HA mice (Henschler et al. 1984). Neither compound produced liver tumors in the mice given daily doses for 18 months.

Stabilizer-free trichloroethylene was administered to male and female F344/N rats at 500 and 1,000 mg/kg for 103 wk (NTP 1990). Reduced survival was observed in male rats and a dose-related increase in hepatic cytomegaly occurred in both sexes. However, no hepatic adenomas or carcinomas were reported. Exposure to trichloroethylene likewise did not produce significant hepatic tumors in four other strains of rat of both sexes administered trichloroethylene by gavage in corn oil for 103 wk at doses from 125 to 2,000 mg/kg (NTP 1988).

Trichloracetic Acid

Trichloroacetic acid is a peroxisome proliferator and a species-specific carcinogen. As shown in Table 4-3, it induces hepatocellular carcinomas when administered in drinking water to male and female B6C3F$_1$ mice (a susceptible mouse strain). Dose-related increases in the incidence of malignant tumors and precancerous lesions have been observed with concentrations in drinking water up to 5 g/L. Significant increases in benign hyperplastic nodules and adenomas were found at concentrations in drinking water as low as 0.35 g/L. However, trichloroacetic acid has not induced significant hepatic tumors in male F344 rats under similar treatment conditions.

A large amount of trichloroacetic acid is formed in susceptible mouse strains after exposure to trichloroethylene (Green and Prout 1985), whereas only a minor portion is found in unresponsive strains of mice (Dekant et al. 1984, 1986b). Saturation of oxidative metabolism of trichloroethylene in rats results in trichloroacetic acid insufficient to induce peroxisome proliferation (Green 1990). Humans, like rats, may exhibit lower rates of oxidation and higher rates of conjugation than do mice.

The carcinogenic potential of peroxisome proliferators, such as trichloroacetic acid, in rodents might be associated with the ability of these agents to increase the rate of hepatocellular proliferation, resulting in hepatocellular hyperplasia and hepatomegaly (Marsman et al. 1988; Popp et al. 1994; Gonzalez et al. 1998). This mode-of-action is discussed later in this chapter.

Dichloroacetic Acid

Dichloroacetic acid, which is metabolized much more rapidly than trichloroacetic acid, is an effective inducer of hepatic tumors in mice and rats (see Table 4-4). It is a major metabolite of trichloroethylene in B6C3F$_1$ mice but is below the limit of detection in similarly dosed Sprague-Dawley rats (Larson and Bull 1992a). Hepatoproliferative lesions increased sharply in male B6C3F$_1$ mice when drinking water concentration increased from 1 to 2 g/L (Bull et al.

TABLE 4-3 Hepatocarcinogenic Effects of Trichloroacetic Acid in Drinking Water Studies with Mice and Rats

			Combined Hyperplastic Nodule and Hepatocellular Adenoma		Hepatocellular Carcinoma		
Species (sex)	Concentration (g/L)	Duration (wk)	Incidence	Tumor/n (multiplicity)	Incidence	Tumor/n (multiplicity)	Reference
B6C3F$_1$ mice (M)	0	61	2/22	0.09	0/22	0	Herren-Freund, et al.
	5	61	8/22	0.5	7/22	0.5	1987
B6C3F$_1$ mice (M)	0	52	1/35	0.03	0/35	0	Bull et al. 1990
	1	52	5/11	0.45	2/11	0.18	
	2	52	15/24	1.04	4/24	0.17	
	2	37	2/11	0.18	3/11	0.27	
B6C3F$_1$ mice (M)	0	60-95	Not reported	Not reported	6.7-10%	0.07-0.15	DeAngelo et al. 1991
	0.05	60	Not reported	Not reported	22%	0.31	
	0.5	60	Not reported	Not reported	38%	0.55	
	4.5	95	Not reported	Not reported	87%	2.2	
	5	60	Not reported	Not reported	55%	0.97	
B6C3F$_1$ mice (F)	0	52	1/40	0.03	0/40	0	Pereira 1996
	0.35	52	6/40	0.15	0/40	0	
	1.2	52	3/19	0.16	0/19	0	
	3.5	52	2/20	0.10	5/20	0.25	
	0	81	2/90	0.02	2/90	0.02	
	0.35	81	14/53	0	0/53	0	
	1.2	81	12/27	0	5/27	0	
	3.5	81	18/18	1.0	5/18	0.28	
F344 rats (M)	0	104	2/23	0.087	0/23	0	Daniel et al. 1993
	0.05	104	2/24	0.083	0/24	0	
	0.5	104	5/20	0.25	0/20	0	
	5	104	1/22	0.045	1/22	0.045	
F344/N rats (M)	0	104	0	0	0	0	DeAngelo et al. 1997
	0.05	104	0	0	0	0	
	0.5	104	0	0	0	0	
	5	104	0	0	0	0	

TABLE 4-4 Hepatocarcinogenic Effects of Dichloroacetic Acid in Drinking Water Studies with Mice and Rats

			Combined Hyperplastic Nodule and Hepatocellular Adenoma		Hepatocellular Carcinoma		
Species (sex)	Concentration (g/L)	Duration (wk)	Incidence	Tumor/n (multiplicity)	Incidence	Tumor/n (multiplicity	Reference
$B6C3F_1$	0	61					Herren-Freund et al. 1987
(M)	5	61	25/26	4.6	21/26	1.7	
$B6C3F_1$	1	52	2/11	0.3	NR	NR	Bull et al. 1990
(M)	2	52	23/24	3.6	5/24	0.25	
	2	37	7/11	2.2	0/11	0	
$B6C3F_1$	0	60	0/10	0	8/12	1.7	DeAngelo et al. 1999
(M)	0.5	60			25/30	2.2	
	3.5	60	12/12	2.3			
	5	60	27/30	2.3			
	0	75	2/28	0.07			
	0.05	75	4/29	0.31			
	0.5	75	3/27	0.11			
	0	104	1/20	0.05	2/20	0.1	Daniel et al. 1992
	0.5	104	12/24	0.5	15/24	0.63	
$B6C3F_1$	0	52	1/40	0.03	0/40	0	Pereira 1996
(F)	0.28	52	0/40	0	0/40	0	
	0.93	52	3/20	0.20	0/20	0	
	2.8	52	7/20	0.45	1/20	0.1	
	0	81	2/90	0.02	2/90	0.02	
	0.28	81	3/50	0.06	0/50	0	
	0.93	81	7/28	0.32	1/28	0.04	
	2.8	81	16/19	5.6	5/19	0.37	
$B6C3F_1$	0	100	14/50	0.25	5/50	0.28	DeAngelo et al. 1999
(M)	0.05	100	11/33	0.5	NR	NR	
	0.5	100	11/24	0.32	5/24	0.68	
	1	100	23/32	0.8	16/32	1.29	
	2	100	13/14	0.85	6/14	2.47	
	3.5	100	8/8	0.64	4/8	2.9	
F344	0	60	0/7	0	0/7	0	DeAngelo et al. 1996
(M)	0.05	60	0/7	0	0/7	0	
	0.5	60	0/7	0	0/7	0	
	2.4	60	26/27	0.96	1/27	0.04	
	0	104	1/23	0.04	0/23	0	
	0.05	104	0/26	0	0/26	0	
	0.5	104	9/29	0.31	2/29	0.1	
	2.4	104	Not done	Not done	Not done	Not done	

1990). Observations of greatly enlarged livers and marked cytomegaly in dichloroacetic-acid-treated mice led to the conclusion that tumorigenesis might depend largely on stimulation of cell division secondary to hepatotoxic damage. A follow-up histologic study to determine the dose relatedness of premalignant hepatic lesions was conducted (Carter et al. 2003) in liver tissues

from male B6C3F$_1$ mice treated in a study by DeAngelo et al. (1999). End points measured were altered hepatic foci, large foci of cellular alternations, adenomas, and carcinomas. Altered hepatic foci, large foci of cellular alterations, and adenomas demonstrated neoplastic progression with time; however, independent of dose and length of exposure, signs of toxicity were also observed in noninvolved liver. The authors interpreted these results as indicating that dichloroacetic acid behaves as a nongenotoxic carcinogen at doses below which genotoxicity has been observed.

In male F344 rats, dichloroacetic acid induced observable signs of toxicity in the nervous system, liver, and myocardium; however, treatment-related neoplastic lesions were observed only in the liver (DeAngelo et al. 1996). The low concentration of dichloroacetic acid in rats exposed to trichloroethylene may explain their relative insensitivity to hepatocarcinogenic effects. Dichloroacetic acid inhibits its own metabolism in the dose range 0.5 to 1 g/L in water; thus, sharp inconsistencies in blood concentrations are seen (ranging from <1 μM to 300-500 μM) (Kato-Weinstein et al. 1998) likely leading to some variation in tumor yield in the mouse and other findings studied in this dose range (Bull et al. 2002).

Chloral Hydrate

Chloral hydrate produces hepatic tumors in male B6C3F$_1$ mice but not in female B6C3F$_1$ mice or in F344 male rats (Table 4-5). A single dose of chloral hydrate at 10 mg/kg administered by intragastric intubation to mice at 15 days of age increased the number of tumors between 48 and 92 wk based on the appearance of three adenomas and three carcinomas among eight animals (Rijhsinghani et al. 1986). Chloral hydrate administered to male B6C3F$_1$ mice for 2 years in drinking water at an average dose of 166 mg/kg/day resulted in a 71% incidence of hepatic tumors (combined adenomas and carcinomas) (Daniel et al. 1992).

In another study, male B6C3F$_1$ mice and male F344/N rats were given chloral hydrate in drinking water for 2 years (George et al. 2000). Time-weighted mean daily doses in rats were 7.4, 37.4, and 162.6 mg/kg/day. No increase in prevalence (percentage of animals with a tumor) or multiplicity (tumors/animal) of hepatocellular tumors was seen in male rats. Time-weighted mean daily doses in mice were 13.5, 65.0, and 146.6 mg/kg/day. Water consumption, survival, and body and organ weights were not altered from control values in any of the chloral hydrate treatment groups for either species. It was concluded that chloral hydrate induced hepatocellular neoplasia in the mouse, with a significant increase in the prevalence of hepatoadenoma and multiplicity at all doses tested and a significant increase in hepatocellular carcinomas in the high-dose group.

A 2-year NTP study in female B6C3F$_1$ mice exposed to chloral hydrate administered in water by gavage was negative for induction of hepatic tumors up to a dose of 100 mg/kg (NTP 2002a). However, a 2-year NTP study in male B6C3F$_1$ mice at the same doses found some evidence of carcinogenic activity based on increased incidences of hepatocellular adenoma or carcinoma (combined) in mice fed ad libitum and increased incidences of hepatocellular carcinoma in dietary-controlled mice (NTP 2002a,b, 2003; Leakey et al. 2003a). Dietary-controlled mice received variably restricted feed allocations to maintain their body weight on a predetermined "idealized" weight curve predictive of a terminal background liver tumor incidence of 15% to 20%. A statistically significant dose response to chloral hydrate was observed in the dietary-controlled animals (terminally adjusted liver tumor incidence 23.4%,

TABLE 4-5 Hepatocarcinogenic Effects of Chloral Hydrate in Mice

				Combined Hyperplastic Nodule and Hepatocellular Adenoma		Hepatocellular Carcinoma		
Species (sex)	Dose (mg/kg)	Exposure Route/Vehicle	Duration (wk)	Incidence	Tumor/n (multiplicity)	Incidence	Tumor/n (multiplicity)	Reference
C57BL ×	0	Oral/drinking	92	0/19	0	2/19	0.11	Rijhsinghani
$C3HF_1$	5	water	92	2/9	0.22	1/9	0.11	et al. 1986
single dose	10		92	3/8	0.38	3/8	0.38	
to neonates								
$B6C3F_1$	0	Oral/drinking	104	1/20	0.05	2/20	0.10	Daniel et al.
	1	water	104	8/24	0.33	11/24	0.46	1992
$B6C3F_1$ (M)	0	Oral/drinking	104	21.4%	0.21	54.8%	0.74	George et al.
	13.5	water	104	43.5%	0.65	54.3%	0.72	2000
	65.0		104	51.3%	0.95	59.0%	1.03	
	146.6 mg/kg/day		104	50.0%	0.72	84.4%	0.72	
$B6C3F_1$ (F)	0	Oral	104	0/37	0	0	0	NTP 2002a
	25	gavage/distilled	104	0/48	0	0	0	
	50	water	104	0/43	0	0	0	
	100		104	0/36	0	0	0	
$B6C3F_1$ (M)	0	Oral	104	12/48	—	4/48	—	NTP 2002b
fed ad	25	gavage/distilled	104	19/48		10/48		
libitum	50	water	104	17/47		10/47		
	100		104	17/48		7/48		
$B6C3F_1$ (M)	0	Oral	104	—	—	16/48[a]	—	NTP 2002b
fed ad	25	gavage/distilled	104			25/48[a]		
libitum	50	water	104			23/47[a]		
	100		104			22/48[a]		
$B6C3F_1$ (M)	0	Oral	104	9/48	—	2/48	—	NTP 2002b;
controlled	25	gavage/distilled	104	7/48		5/48		Leakey et al.
dietary	50	water	104	10/48		4/48		2003a
intake	100		104	10/48		8/48		

[a]Adenoma or carcinoma.

23.9%, 29.7%, and 38.6% for the four dose groups) but not the test groups fed ad libitum (terminally adjusted liver tumor incidence 33.4%, 52.6%, 50.6%, and 46.2%) (Leakey et al. 2003b). Dietary control was deemed to improve survival and decrease interassay variation.

Overall, chloral hydrate appears to be a species-, strain-, and sex-specific weak carcinogen. Furthermore, because two of the metabolites of chloral hydrate are trichloroacetic acid and dichloroacetic acid, it is difficult to assess the direct contribution of chloral hydrate to the specific carcinogenic effects observed solely in male $B6C3F_1$ mice.

Collective Assessment of Animal Data

Trichloroacetic acid and chloral hydrate appear to be capable of inducing liver tumors only in mice, but dichloroacetic acid also induces liver tumors in rats. The blood concentration of trichloroacetic acid required to induce liver cancer in mice approaches the millimolar range; trichloroacetic acid is a peroxisome proliferator in the same dose range that induces liver cancer. The concentration of dichloroacetic acid associated with liver cancer is in the submicromolar range (Kalkuhl et al. 1998). The weak carcinogenicity of chloral hydrate is largely due to its metabolic conversion to trichloroacetic acid or dichloroacetic acid.

Altered gene expression in specific genes involved in the functional categories of cell growth, tissue remodeling, apoptosis, cancer progression, and xenobiotic metabolism has been observed in mouse liver after administration of a tumorigenic dose of dichloroacetic acid at 2 g/L in drinking water for 4 wk (Thai et al. 2003). Dichloroacetic acid produces tumors in mice that display immunoreactivity to a c-Jun antibody, whereas trichloroethylene-induced tumors do not show this antibody reactivity (Stauber and Bull 1997). More recent work, in which trichloroacetic acid and dichloroacetic acid were given to mice alone and in various dose combinations, showed that dichloroacetic acid and trichloroacetic acid produced some tumors that were c-Jun^+, and many that were c-Jun^-; the number with a mixed phenotype increased with the relative dose of dichloroacetic acid (Bull et al. 2002). Mutation frequency of the H-*ras* protooncogene in mouse tumors induced by trichloroacetic acid alone was significantly different from that observed in trichloroethylene-induced tumors (0.44 versus 0.21), but that observed with dichloroacetic-acid-induced tumors (0.33) was not significantly different from that observed with trichloroethylene. No significant difference was observed in mutation spectra of tumors produced by the three compounds. Thus, dichloroacetic acid appears to produce liver tumors with a different phenotype than those produced as a result of trichloroacetic acid exposure. Dichloroacetic acid also induces markedly enlarged livers associated with cytomegaly (Bull 2000).

Both trichloroacetic acid and dichloroacetic acid are effective as rodent liver carcinogens at doses that do not produce cytotoxicity. Trichloroacetic acid produces liver tumors in mice with a phenotype common to peroxisome proliferators, whereas dichloroacetic acid increases the growth of liver tumors and produces tumors with a phenotype distinct in several aspects from those produced by trichloroacetic acid. An initiation-promotion study of each compound alone or in pairwise combinations of trichloroacetic acid, dichloroacetic acid, and carbon tetrachloride was conducted in male $B6C3F_1$ mice (Bull et al. 2004). Carbon tetrachloride was chosen for its ability to promote growth of liver tumors through cytotoxicity, producing a reparative hyperplasia growth stimulus. Thus, trichloroacetic acid, dichloroacetic acid, and carbon tetrachloride were hypothesized to have individually different modes of action as promoters. In

general, interactions between carbon tetrachloride and trichloroacetic acid were seen to be additive and likely acting via different mechanisms whereas interactions between carbon tetrachloride and dichloroacetic acid were generally less than additive with a consistent dose-dependent decrease in the growth rate of tumors promoted by carbon tetrachloride. Dichloroacetic acid appears to exert an inhibitory effect on the growth of trichloroacetic-acid-promoted tumors. Thus, interactions were inhibitory or additive, but there appeared to be no evidence of synergy.

Differences between mice and rats in the development of hepatocellular adenoma and carcinoma from trichloroethylene and its metabolites is consistent with other non-mutagenic compounds and is not particularly useful for determination of mechanism of action or extrapolation to humans. This is due to controversy surrounding the validity of results in mouse liver resulting from the large number of non-mutagens that induce such tumors and the high and variable spontaneous tumor rates in some strains. Gold and Sloane (1995) examined the Carcinogenic Potency Database where 174 chemicals were evaluated as liver carcinogens in rats and mice. More mutagens than non-mutagens have been identified as liver carcinogens in each species (in mice 84 mutagens and 70 non-mutagens; in rats 75 mutagens and 32 non-mutagens). Their analysis indicated a species difference in the predominance of liver cancer in mice compared to rats. Among chemicals with positive results in the mouse, 55% (84/154) of mutagens compared to 71% (70/99) of non-mutagens induce liver tumors, while the proportions among positive chemicals in the rat are 39% (75/194) and 33% (32/98). Thus, while the proportion of rat carcinogens that are positive in the liver is similar for mutagens and non-mutagens, a higher proportion of non-mutagenic mouse carcinogens are positive in the liver than mutagenic carcinogens. This finding in mice reflects that chlorinated compounds (composed solely of chlorine, carbon, hydrogen, and, optionally, oxygen) are frequently positive in the mouse liver and are not mutagenic. Excluding the chlorinated compounds, results in mice are similar for mutagenic and non-mutagenic carcinogens; 56% (79/142) of mutagens and 59% (40/68) of non-mutagens are mouse liver carcinogens. In the Carcinogenic Potency Database, 261 rodent carcinogens have been tested in both rats and mice, and 82 (31%) induce tumors in only one target site of one species. The mouse liver is the most common single-site, single-species target organ for both mutagens (12 chemicals) and non-mutagens (19 chemicals). Many of the non-mutagens in this group are chlorinated compounds. Thus, the species difference in the potency of trichloroethylene and its metabolites to induce liver tumors must be put in context with this historical data.

Human Studies

Because it was not possible for the committee to provide a comprehensive evaluation of the epidemiologic evidence on trichloroethylene and different cancers, it borrowed a previously compiled summary of the epidemiologic evidence on liver cancer from the Institute of Medicine (IOM 2003) to give some perspective on the evidence for liver cancer (see Table 4-6). The list was updated with one study published since the IOM report. Some common limitations of the studies that were reviewed include a relatively small number of cases of liver cancer and a lack of control for potential confounding by risk factors such as alcohol consumption and hepatitis B (see methodology and exposure information on some of the specific studies reviewed in Chapter 3, Tables 3-4 and 3-6). Another issue is that some studies reported findings for primary liver

TABLE 4-6 Selected Epidemiologic Data on Liver Cancer or Hepatobiliary Cancers and Exposure to Trichloroethylene

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Cohort Studies—Incidence			
Raaschou-Nielsen et al. 2003	Workers in Denmark		
	Males		
	All TCE-exposed workers	27[a]	1.1 (0.7-1.6)
	<1 yr employed	9	1.3 (0.6-2.5)
	1-4.9 yr employed	9	1.0 (0.5-1.9)
	≥5 yr employed	9	1.1 (0.5-2.1)
	Females		
	All TCE exposed workers	7	2.8 (1.1-5.8)
	<1 year employed	2	2.8 (0.3-10.0)
	1-4.9 yr employed	4	4.1 (1.1-10.5)
	≥5 yr employed	1	1.3 (0.0-7.1)
Morgan and Cassady 2002	Redlands, CA, community exposed to TCE in drinking water	28[a]	1.29 (99% CI 0.74-2.05)
Hansen et al. 2001	Biologically monitored Danish workers		
	Males	5[b]	2.6 (0.8-6.0)
	Females	0	—
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Males		
	No exposure	1[a]	0.8 (0.1-12.0)
	<5 unit-yr	2	1.2 (0.1-13.8)
	5-25 unit-yr	1	1.0 (0.1-16.0)
	>25 unit-yr	3	2.6 (0.3-25.0)
Antilla et al. 1995	Biologically monitored workers in Finland		
	Entire period since first measurement	5[a]	2.27 (0.74-5.29)
	0-9 yr	0	— (0.0-6.59)
	10-19 yr	2	1.74 (0.21-6.29)
	≥20 yr	3	6.07 (1.25-17.7)
	Mean personal U-TCA level		
	<100 μmol/L	2	1.64 (0.20-5.92)
	100+ μmol/L	2	2.74 (0.33-9.88)
Axelson et al. 1994	Biologically monitored Swedish workers	4[a]	1.41 (0.38-3)
Cohort Studies—Mortality			
Chang et al. 2003	Electronics-manufacturing workers in Taiwan[c]		
	Males	0	0.00 (NA)
	Females	0	0.00 (NA)
Boice et al. 1999	Aircraft-manufacturing workers in California		
	All exposed factory workers employed at least 1 yr since 1960 with routine exposure	4[b]	0.54 (0.15-1.38)
	Duration of potential exposure (routine or intermittent)		
	<1 year exposed	4[a]	0.53 (0.18-1.60)
	1-4 yr exposed	3	0.52 (0.15-1.79)
	≥5 yr exposed	6	0.94 (0.36-2.46)

TABLE 4-6 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Ritz 1999	White male U.S. uranium-processing workers		
	TCE, cutting fluids, or kerosene	8[a]	1.66 (0.71-3.26)
	TCE, light exposure		
	>2 yr, no latency	3	0.93 (0.19-4.53)
	>2 yr, 15-yr latency	3	1.16 (0.24-5.60)
	>5 yr, no latency	3	1.90 (0.35-10.3)
	>5 yr, 15-yr latency	3	2.86 (0.48-17.3)
	TCE, moderate exposure		
	>2 yr, no latency	1	4.97 (0.48-51.1)
	>2 yr, 15-yr latency	1	5.53 (0.54-56.9)
	>5 yr, no latency	1	8.82 (0.79-98.6)
	>5 yr, 15-yr latency	1	12.1 (1.03-144)
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Primary liver cancer for all TCE exposed	4	1.7 (0.2-16.2)
	Liver and biliary cancer by cumulative TCE exposure		
	Males		
	No exposure	3	0.5 (0.1-2.4)
	<5 unit-yr	6	1.1 (0.3-4.1)
	5-25 yr	3	0.9 (0.2-4.3)
	>25 unit-yr	3	0.7 (0.2-3.2)
	Females		
	No exposure	3	4.2 (0.7-25.0)
	<5 unit-yr	1	1.6 (0.2-18.2)
	5-25 unit-yr	0	—
	>25 unit-yr	2	2.3 (0.3-16.7)
Morgan et al. 1998	Aerospace workers in Arizona		
	TCE-exposed subcohort:	6[b]	0.98 (0.36-2.13)
	Low cumulative exposure	3	1.32 (0.27-3.85)
	High cumulative exposure	3	0.78 (0.16-2.28)
	Peak and cumulative exposure[c]:		
	Peak: medium and high versus low and no exposure	3 [a]	0.98 (0.29-3.35)
	Cumulative (low)	3	2.12 (0.59-7.66)
	Cumulative (high)	3	1.19 (0.34-4.16)
Greenland et al. 1994	White male transformer-assembly workers, ever exposed	NA	0.54 (0.11-2.63)
Garabrant et al. 1988	Aircraft-manufacturing workers, San Diego (about 37% of jobs had exposure to TCE)	8	0.94 (0.40-1.86)
Case-control—Mortality			
Lee et al. 2003	Community downstream of an electronics factory in Taiwan	53	2.57 (1.21-5.46)

[a]Results are for primary liver cancer.
[b]Results are for liver and biliary cancer combined.
[c]Internal cohort analyses for peak and cumulative exposure to trichloroethylene classifications used Cox proportional-hazards models.
Abbreviations: NA, not available; CI, confidence interval: TCE, trichloroethylene; U-TCA, urinary trichloroacetic acid.
Source: Adapted from IOM 2003.

cancer, and others reported findings for biliary and primary liver cancer combined. This could result in misclassification of the outcome if these two cancer sites (liver and biliary) are etiologically distinct with respect to the effects of trichloroethylene exposure. In addition, only large cohort studies would have adequate statistical power to estimate excess risks and exposure-response relationships, as the incidence of liver cancer in the United States is low; the age-adjusted rate of cancer of the liver and intrahepatic bile duct is 6 per 100,000 people (SEER 2005). The American Cancer Society (2006) estimates that approximately 18,500 people will be diagnosed liver and intrahepatic bile duct cancer in 2006.

Cohort Studies

Excess incidence of liver cancer was observed in most cohort studies that specifically examined exposures to trichloroethylene (Axelson et al. 1994; Antilla et al. 1995; Hansen et al. 2001; Morgan and Cassady 2002; Raaschou-Nielsen et al. 2003). These findings were generally based on a small number of incident cases and thus were statistically unstable. Only one study reported a statistically significant excess of liver cancer incidence for the entire cohort (Raaschou-Nielsen et al. 2003). An excess among women (relative risk [RR] = 2.8; 95% confidence interval [CI] = 1.1, 5.8), but not among males (RR = 1.1, 95% CI = 0.7, 1.6), was reported in this study. Liver cancer incidence among females was significantly increased among women with 1 to 4.9 years of exposure (RR = 4.1, 95%CI = 1.1, 10.5). The incidence of liver cancer was not significantly elevated in the highest exposure group (RR = 1.3, 95%CI = 0.0,7.1); however, there was only one case and less than one case expected in this group and, thus, the findings were highly unstable for this group. Evidence for an exposure-response relationship between the incidence of liver cancer and trichloroethylene exposure was also observed in the study by Antilla et al. (1995), who reported a statistically significant excess of liver cancer incidence in their highest duration of exposure category (>20 years; RR = 6.07, 95% CI = 1.25, 17.7).

Findings from the cohort studies that reported findings for mortality were mixed, with one study reporting no difference (Garabrant et al. 1988), three studies reporting a deficit (Greenland et al. 1994; Morgan et al. 1998; Boice et al. 1999), and two studies reporting an excess (Blair et al. 1998; Ritz 1999) in deaths from liver cancer. One study (Ritz 1999) found evidence of an exposure-response relationship; mortality from liver cancer was found to increase with degree (light versus moderate) and duration of exposure and time since first exposure (>15 years). A statistically significant excess of liver cancer (RR = 12.1) was reported among workers with moderate exposure, greater than 5 years of exposure, and at least 15 years since the first exposure; this finding was based on only one case and thus was not statistically stable (95% CI = 1.03, 144).

Case-Control Studies

A strength of the case-control studies is that they can have greater statistical power than cohort studies for evaluating rare outcomes such as liver cancer, but the power also depends on the prevalence of the exposure of interest, which is often low in general populations. A frequent weakness of population-based case-control studies is their inability to reliably document and

estimate workplace exposures. IOM (2003) identified four case-control studies of liver cancer and exposure to organic solvents in general (Stemhagen et al. 1983; Hardell et al. 1984; Hernberg et al. 1988; Heinemann et al. 2000). One study has been published since then in which exposure to trichloroethylene was investigated. Lee et al. (2003) conducted a population-based case-control study in a Taiwanese village downstream from an electronic factory that contaminated community wells with trichloroethylene, tetrachloroethylene, and 1,1-dichloroethylene. Trichloroethylene concentrations in the well water were reported to be an order of magnitude higher than those for tetrachloroethylene and 1,1-dichloroethylene. This study reported increased mortality odds ratios among males for all cancer and for liver cancer for the periods after 10 years of latency—namely, 1980-1989 and 1990-1997. The adjusted mortality odds ratios for liver cancer in males was 2.6 (95% CI = 1.2, 5.5), with a significant linear trend for the period effect. This study did not address potential confounding related to hepatitis viral infection status, a risk factor for liver cancer, or potential misclassification due to the inclusion of secondary liver cancer among the case series.

Mode of Action

A number of modes of action have been proposed for the carcinogenic action of trichloroethylene and its metabolites in the liver, including genotoxicity, mutagenicity activation of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), and alterations in cellular signaling pathways. This section reviews the available evidence for each of these modes of actions and the relevance to humans. Although these modes of action are discussed separately, it is likely that multiple modes of action are involved in the carcinogenic process.

Mutagenicity and Genotoxicity

Mutagenicity refers to the ability of a chemical to induce heritable mutations (damage that can pass to daughter somatic cells), whereas genotoxicity is a broader term that includes mutational end points, cytogenetic analysis, and primary DNA damage.

Most mutagenicity assays for trichloroacetic acid, dichloroacetic acid, and chloral hydrate are negative. Dichloroacetic acid and trichloroacetic acid do not consistently induce DNA damage in the livers of mice treated with hepatotoxic doses (IARC 1995a). The weight of evidence on the mutagenicity of chloral hydrate, dichloroacetic acid, and trichloroacetic acid indicates that a chemically induced mutation is unlikely to be a key event in the induction of tumors (Moore and Harrington-Brock 2000). In general, these chemicals require very high doses to elicit positive results, principally in in vitro tests. For example, chloral hydrate was positive in approximately 10 in vitro genotoxicity studies; however, in vivo results were mixed. Moreover, the potency of chloral hydrate in these studies was very low. Dichloroacetic acid has been the most extensively studied and has shown positive results in the standard Ames test protocol and in vitro mouse lymphoma assay; it was shown to induce very small increases in mouse bone marrow micronuclei and to increase DNA strand breaks in mouse and rat liver cells in vivo. However, DNA damage assays do not prove that a chemical can cause mutational damage. The collective evidence indicates that dichloroacetic acid is likely mutagenic but very weakly so. Trichloroacetic acid is the least mutagenic of the three metabolites, being negative in the

Salmonella test and only weakly positive in the mouse lymphoma assay. It is unlikely that trichloroacetic acid would contribute to tumor formation through a mutational mechanism.

Neonatal B6C3F_1 mice were administered chloral hydrate, trichloroacetic acid, and trichloroethylene by intraperitoneal injection at 8 and 15 days of age (Von Turgeln et al. 2002). At 12 months, only male mice treated with the positive control compounds had significant induction of liver tumors. Additional male mice were treated as above and livers were excised 24 and 48 hours and 7 days after the final dose. At 24 and 48 hours, mice treated with chloral hydrate or trichloroacetic acid showed significantly higher 8-oxo-2′deoxyguanosine formation, indicating increased endogenous DNA adduct formation through lipid peroxidation or oxidative stress; the authors concluded that neonatal B6C3F_1 male mice are not sensitive to chloral hydrate or trichloroacetic acid as liver carcinogens. DNA and insulin-like growth factor II were demonstrated to be hypomethylated in mouse liver tumors in an initiation-promotion experiment (Tao et al. 2004b). DNA in both dichloroacetic-acid- and trichloroacetic-acid-promoted tumors was shown to be hypomethylated. Specific genes involved in several functional categories, including cell growth, tissue remodeling, apoptosis, cancer progression, and xenobiotic metabolism, were shown to have altered gene expression in dichloroacetic-acid-induced mouse liver tumors (Thai et al. 2003). Overall, the evidence indicates that none of the three metabolites under consideration here is likely to act principally by a mutational or genotoxic mechanism as liver carcinogens.

Peroxisome Proliferator-Activated Receptor Agonism

Peroxisome proliferators are a class of compounds that when fed to laboratory animals result in liver cancer (see Appendix E for detail and perspective). A key mode of action in this carcinogenic process is activation of the nuclear receptor PPARα. The human relevance of PPAR agonism is a subject of debate in the scientific community that has resulted in at least two important working groups and subsequent publications (Klaunig et al. 2003; IAS 2005). Several review articles have detailed the role of peroxisome proliferators and PPARs in the carcinogenic process (Green 1992, 1995; Green and Wahli 1994; Cattley et al. 1995; James et al. 1998; Gelman et al. 1999; Vanden Heuvel 1999a,b; Bull 2000; Corton et al. 2000a,b; Yeldandi et al. 2000; Melnick 2001; Guan 2002; Youssef and Badr 2002, 2005; Yu et al. 2003; Zhao and Jiang 2003; Kennedy et al. 2004; Lai 2004; Bosgra et al. 2005; Corton and Lapinskas 2005; O'Brien et al. 2005). Trichloroethylene, trichloroacetic acid, and dichloroacetic acid are considered peroxisome proliferators and they induce morphologic and biochemical changes that typify this class of chemicals. Chloral hydrate is considered either a very weak or a nonperoxisome proliferator. Thus, at least in terms of trichloroethylene, trichloroacetic acid, and dichloroacetic acid, the PPARα agonism (i.e., peroxisome proliferation) mode of action is a viable possibility which will be examined in more detail herein. The general applicability of PPARα agonism to human health is discussed in Appendix E.

Peroxisome Proliferators and Liver Cancer

The proposed mode of action for peroxisome proliferators is depicted in Figure 4-1, which shows several key events that ultimately result in rodent liver tumors. First, peroxisome

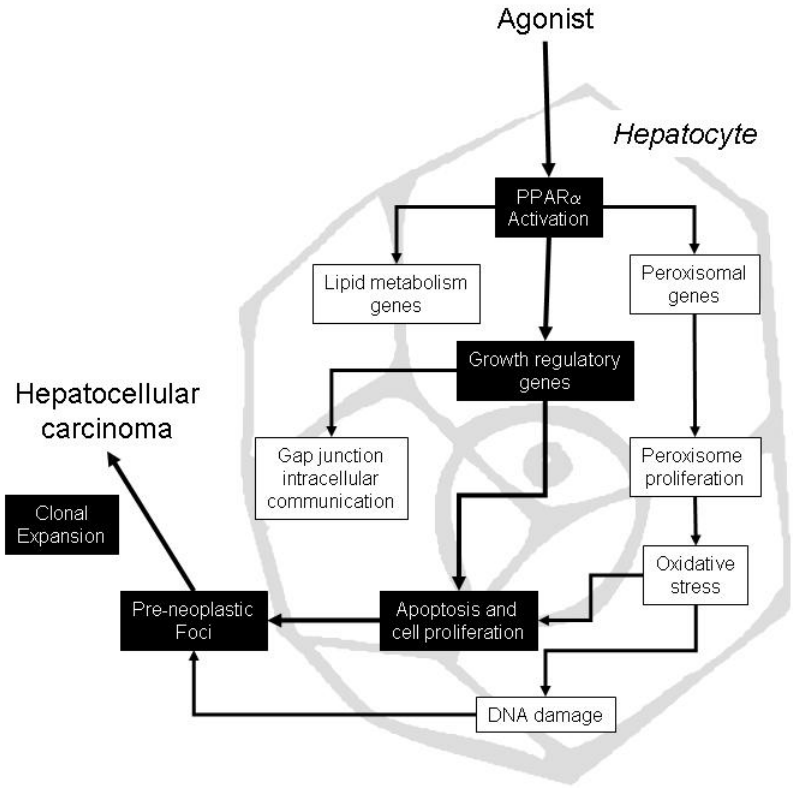

FIGURE 4-1 Proposed mode of action for liver tumor formation by peroxisome proliferators. Events causally related to adenoma or carcinoma formation are shown in black boxes; associated events are in white boxes. Source: Adapted from Klaunig et al. 2003.

proliferators activate PPARα, which regulates the transcription of genes involved in peroxisome proliferation, cell cycle and apoptosis, and lipid metabolism. Alterations in growth regulatory genes lead to perturbations in cell proliferation and apoptosis. Suppression of apoptosis coupled with stimulation of cell proliferation allows DNA-damaged cells to persist and proliferate, giving rise to preneoplastic foci and ultimately to tumors via further clonal expansion. Peroxisome proliferation per se is considered to be evidence of PPARα activation but it might or might not be related to the tumor formation. However, peroxisome proliferation could lead to oxidative stress, which could contribute to the mode of action by causing indirect DNA damage or by contributing to the stimulation of cell proliferation. As with other tumor promoters, PPARα ligands also inhibit gap junction intercellular communication, an event associated with increased cell proliferation. Several peroxisome proliferators stimulate nonparenchymal hepatic Kupffer cells and these resident macrophages affect the cell proliferation; this event is not depicted in Figure 4-1 because the importance of this event is controversial and no studies have been performed in this regard with trichloroethylene. The weight of evidence for the causally related events is discussed elsewhere (Klaunig et al. 2003; IAS 2005).

A minimal set of data elements to support a convincing demonstration that rodent liver tumors have arisen as a result of a PPARα mode of action would consist of PPARα agonism combined with light- or electron-microscopic evidence for peroxisome proliferation. Alternatively, evidence for PPARα agonism (in a receptor assay) combined with increases in

liver weight and one (induction of acyl CoA oxidase) or more (e.g., induction of CYP4A) of the specific in vivo markers of peroxisome proliferation would suffice. Demonstration that liver growth was accompanied by at least transiently increased rates of replicative DNA synthesis or decreased apoptosis also would significantly strengthen the case. The most convincing information showing that a particular compound induces liver cancer by PPARα mode of action would be through the use of the null mouse model. If the compound induces tumors in the wildtype, but not the PPARα$^{-/-}$ mouse, then this mode of action would be verified. Short of this information, the minimal criteria listed above (PPARα agonism with accompanying altered proliferation and growth characteristics) would be considered highly supportive. The absence of liver tumors in PPARα-null mice would definitively demonstrate the role of PPARα. Whether trichloroethylene and its metabolites meet this minimal data set is discussed below.

Trichloroethylene. The PPARα mode of action relative to trichloroethylene is summarized in Table 4-7; many of these effects might be attributable to trichloroethylene metabolites—in particular, trichloroacetic acid and dichloroacetic acid. Trichloroethylene activates mouse and human PPARα, albeit at high concentrations (1 mM), and can regulate known target genes for PPARα (Maloney and Waxman 1999; Nakajima et al. 2000; Laughter et al. 2004). Recent studies in PPARα null mice have shown that several events depend on this protein, including regulation of peroxisomal enzymes, cell proliferation, and perhaps some cell cycle regulatory genes (Klaunig et al. 1991; Stauber and Bull 1997; Tao et al. 1999, 2000b; Laughter et al. 2004). To the committee's knowledge, a long-term bioassay in this mouse model system has not been performed. An important characteristic of all tumor promoters is their ability to selectively enhance survival of a particular phenotype of foci that ultimately gains a growth advantage. The work of Stauber and Bull (1997) and Bull et al. (2002) examining oncogene expression and mutations as well as that Tao et al. (1999, 2000a,b) examining DNA methylation have provided substantial information on the tumor phenotypes in trichloroacetic-acid- and dichloroacetic-acid-treated rodents, although the data for trichloroethylene are much less extensive. However, trichloroethylene causes tumors that are mixed for c-*jun* expression but consistently contain codon 61 mutations in c-Ha-*ras*. Interestingly, the tumor phenotypes of trichloroethylene-, trichloroacetic-acid, and dichloroacetic-acid-induced tumors are not identical.

The species difference in tumor-promoting effects between rats and mice can be examined relative to the PPARα mode of action. The species difference in sensitivity to palmitoyl CoA oxidation activity was studied in F344 rats after treatment with trichloroethylene, tetrachloroethylene, and trichloroacetic acid (Goldsworthy and Popp 1987), and in Osborne-Mendel and Alderly Park rats and B6C3F$_1$ and Alderley Park mice treated with trichloroethylene (Elcombe et al. 1985). The data indicate that rats treated with trichloroethylene (or tetrachloroethylene) do not show increases in peroxisomal enzyme activities, whereas rats treated with trichloroacetic acid have significant increases in peroxisomal enzyme activity. On the other hand, mice responded to treatment with trichloroethylene, exhibiting increases in peroxisomal volume density and induction of peroxisomal enzyme activities, catalase, and palmitoyl CoA oxidation. The accepted explanation for this species difference in sensitivity is a difference in metabolism. Trichloroethylene is metabolized by cytochrome P-450s and other noncytochrome P-450 oxidative enzymes to trichloroacetic acid and dichloroacetic acid. Goldsworthy and Popp (1987) clearly demonstrated that trichloroacetic acid was a peroxisome proliferator in rats. Yet rats metabolize trichloroethylene more slowly than mice and metabolism appears to be saturable

TABLE 4-7 Trichloroethylene and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Human and mouse PPARα activated in transient transfection reporter assays. Studies from PPARα null mice show that the effects on cell proliferation and peroxisome proliferator target genes are PPARα dependent.	Maloney and Waxman 1999; Nakajima et al. 2000; Laughter et al. 2004
Regulation of growth regulatory genes	Increased c-jun and c-myc mRNA levels in nontumor tissue. Several potential growth regulatory target genes examined using microarrays showing a PPARα-dependent response.	Tao et al. 1999, 2000b; Laughter et al. 2004
Cell proliferation or apoptosis	Although there is no or little increase in hepatocyte labeling index in rats, mice exposed to trichloroethylene have higher rates of cell proliferation. This event is PPARα dependent. Trichloroethylene inhibited intercellular communication in mouse hepatocytes and not in rat hepatocytes.	Klaunig et al. 1989; 1991; Stauber and Bull 1997; Laughter et al. 2004
Clonal expansion	Tumors that arise from trichloroethylene treatment are basophilic with a relatively consistent mutational spectrum (c-Ha-*ras* codon 61).	Bull et al. 2002
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Increased peroxisomes and peroxisomal enzymes are seen in mice but much less in rats. Increases in CN-insensitive palmitoyl CoA oxidation is induced by trichloroethylene but to a lesser extent than fibrates. ACO and CYP4A induction is PPARα dependent.	Goldsworthy and Popp 1987; NTP 1988; Nakajima et al. 2000; Laughter et al. 2004
Oxidative stress	Increased thiobarbituric acid reactive substances and decreases in reduced glutathione in mouse liver.	Watanabe and Fukui 2000

(Green and Prout 1985; Prout et al. 1985; Green et al. 1997a). Thus, the lack of tumorigenesis in rats compared with mice can be explained by a difference in metabolism of the active metabolite.

Trichloroacetic Acid. Trichloroacetic acid is most often cited as the hepatocarcinogenic metabolite of trichloroethylene. Thus, much of the same evidence for a PPARα mode of action for trichloroethylene could be provided for trichloroacetic acid. However, as outlined in Table 4-8, there are some differences in the strength of the data relative to trichloroethylene. There have been no studies showing the PPARα dependence on cell proliferation induced by trichloroethylene, although hypertrophy was not seen in PPARα null mice. A more extensive characterization of trichloroacetic-acid-induced tumors is available that clearly shows clonal

TABLE 4-8 Trichloroacetic Acid and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Mouse PPARα activated in transient transfection reporter assays with mixed results regarding human PPARα. Studies from PPARα null mice show that effects on peroxisome proliferator target genes are PPARα dependent.	Maloney and Waxman 1999; Walgren et al. 2000a,b; Laughter et al. 2004
Regulation of growth regulatory genes	Has not been studied.	
Cell proliferation or apoptosis	Increased labeling index and dose-dependent increases in cell proliferation were observed. Hypertrophy was noted in the PPARα wild-type but not PPARα null mice. Trichloroacetic acid inhibited intercellular communication in mouse hepatocytes but not in rate hepatocytes.	Stauber and Bull 1997; Klaunig et al. 1989; Ge et al. 2001; Laughter et al. 2004
Clonal expansion	Increased clonal expansion of tumors that resemble those seen by peroxisome proliferators (and unlike DCA). Basophilic foci lacking GST-pi. See spectrum of mutations in K- and H-*ras*. Expansion not due to cytotoxicity. DNA hypomethylation is seen in TCA-induced tumors in the promoters of c-*myc*, IGF-II, and c-*jun*.	Ferreira-Gonzalez et al. 1995; Pereira 1996; Pereira and Phelps 1996; Pereira et al. 1997, 2001; Tao et al. 1996, 2004b; Latendresse and Pereira 1997; Bull et al. 2004
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Increases in peroxisomal enzymes are seen in mouse and to a lesser extent in rat liver. CYP4A induction is PPARα dependent.	Goldsworthy and Popp 1987; Odum et al. 1988; Walgren et al. 2004
Oxidative stress	Has not been studied.	

Abbreviations: DCA, dichloroacetic acid; GST, glutathione *S*-transferase; IGF-II, insulin-like growth factor II; TCA, trichloroacetic acid.

expansion occurs as a result of treatment with this chemical (greater evidence than provided by trichloroethylene).

Dichloroacetic Acid. Although the initial events in the PPARα mode of action associated with dichloroacetic acid seem similar to those of trichloroethylene and trichloroacetic acid, there are some intriguing differences (see Table 4-9). For example, although dichloroacetic acid can activate PPARα (perhaps slightly less than trichloroacetic acid does) and cause peroxisome proliferation, the liver hypertrophy is not PPARα dependent. Also, the clonal expansion of preneoplastic foci by dichloroacetic acid is quite different with different phenotypic and genetic markers than by trichloroacetic acid and trichloroethylene.

Chloral Hydrate. Although chloral hydrate is used medically as a sedative or hypnotic and as a rubefacient in topical preparations, it has not been studied extensively in terms of the PPARα mode of action (see Table 4-10). The most extensive study was performed by the

TABLE 4-9 Dichloroacetic Acid and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Mouse PPARα activated in transient transfection reporter assays with mixed results regarding human PPARα. Studies from PPARα null mice show that the effects on peroxisome proliferator target genes are PPARα dependent.	Maloney and Waxman 1999; Walgren et al. 2000a,b; Laughter et al. 2004
Regulation of growth regulatory genes	Has not been studied.	
Cell proliferation or apoptosis	Increased labeling index and dose-dependent increases in cell proliferation were observed. Hypertrophy was noted in the PPARα wild-type and PPARα null mice.	Stauber and Bull 1997; DeAngelo et al. 1999; Walgren 2000a,b; Ge et al. 2001; Laughter et al. 2004
Clonal expansion	Increased clonal expansion of tumors that do not resemble those seen by peroxisome proliferators (and unlike TCA and trichloroethylene). Eosinophilic foci positive for GST-pi, TGF-α, c-*jun*, and c-*myc* and negative for c-*fos*. DNA hypomethylation is seen in DCA-induced tumors in the promoters of c-*myc* and IGF-II. This hypomethylation and tumor formation can be reversed by methionine (although peroxisome proliferation may not be). Reversal of hypomethylation can be reversed after removal of DCA (unlike TCA). Loss of heterozygosity is observed in DCA-induced tumors.	Anna et al. 1994; Ferreira-Gonzalez et al. 1995; Pereira 1996; Pereira and Phelps 1996; Pereira et al. 1997, 2004; Tao et al. 1996, 2004b; Latendresse and Pereira 1997; Miller et al. 2000; Carter et al. 2003
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Increases in peroxisomal enzymes are seen in mouse and to a lesser extent in rat liver. CYP4A induction is PPARα dependent.	Everhart et al. 1998; DeAngelo et al. 1999; Pereira et al. 2004; Walgren 2004

Abbreviations: DCA, dichloroacetic acid; GST, glutathione *S*-transferase; IGF-II, insulin-like growth factor II; TCA, trichloroacetic acid; TGF-α, transforming growth factor type α.

National Toxicology Program (NTP 2002b). Groups of male mice received chloral hydrate in distilled water by gavage at doses of 25, 50, or 100 mg/kg 5 days per week for 104 to 105 wk. Each dose group was divided into two dietary groups of mice. The mice fed ad libitum had free access to feed, and the diet-controlled mice received feed in measured daily amounts calculated to maintain body weight on a previously computed idealized body weight curve. Chloral hydrate did not significantly induce either lauric acid 4-hydroxylase activity or CYP4A immunoreactive protein in any of the dosed groups of mice fed ad libitum. However, the high dose significantly induced both lauric acid 4-hydroxylase activity and CYP4A immunoreactive protein in the diet-controlled mice. Moreover, the induction-response profile of CYP4A was similar to the increase in the incidence of liver neoplasms at 2 years in the diet-controlled mice, with the major effect occurring in the 100-mg/kg group.

Weight of Evidence

Table 4-11 summarizes the committee's evaluation of the weight of evidence for a PPARα mode of action in rodents for trichloroethylene and its metabolites. The assessment is based on the general framework described by Cohen et al. (2003) and illustrated for PPARα agonists by Klaunig et al. (2003). Briefly, strong weight of evidence is defined as several studies that support the mode of action, and weak weight of evidence is defined by having a single study from a single laboratory or a significant amount of contradiction in the literature.

Dose-Response

The dose-response relationships for the key events in the PPARα mode of action are shown in the Table 4-12. Note the results from the comparison of wild-type with PPARα null mice that show the role of this receptor in the toxicity of trichloroethylene or its metabolites (discussed earlier in this chapter). PPARα activation per se is reserved for trans-activation assays, as that is the most direct and definitive way to examine nuclear receptor agonism.

TABLE 4-10 Chloral Hydrate and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Has not been studied.	
Regulation of growth regulatory genes	Has not been studied.	
Cell proliferation or apoptosis	Although hepatocelluar carcinoma was observed in mice and not rats, there was no increase in cell proliferation in either species. Chloral hydrate had no effect on hepatocyte intercellular communication in either rat or mouse cells.	Klaunig et al. 1989; George et al 2000
Clonal expansion	Has not been studied.	
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Although hepatocellular carcinoma was observed in mice and not rats, there was no increase in palmitoyl CoA oxidation in either species. In diet-controlled mice, peroxisome proliferation and an increase in CYP4A protein and enzyme activity were seen.	George et al 2000; NTP 2002b
Oxidative stress	Has not been studied.	

TABLE 4-11 Strength of the Weight of Evidence for PPARα Mode of Action for Trichloroethylene and Its Metabolites

Chemical	Weight of Evidence[a]	Comments
TCE	Strong	TCE activates PPARα at high concentrations and regulates a variety of target genes. Studies with PPARα null mice show that most responses are dependent on this receptor, including peroxisome proliferation, cell proliferation, and target gene expression. More evidence could be provided by a long-term bioassay in this model system.
TCA	Strong	TCA activates PPARα at high concentrations. Less extensive characterization of PPARα dependence on cell proliferation is provided than is known for TCE. However, the evidence of clonal expansion and phenotypic characteristics of tumors is strong and shows similarity to peroxisome proliferators.
DCA	Strong	DCA activates PPARα at high concentrations. As is the case with TCA, PPARα dependence of DCA's effects on cell proliferation is less than for trichloroethylene. Increasingly, it appears that DCA-induced clonal expansion is dissimilar to that of TCA and TCE. The reason for this discrepancy is not known but may require examination of tumors from PPARα null mice. Also, liver weight changes (and presumably cell proliferation) are not dependent on PPARα, indicating a potential for other modes of action that would be different than that of TCA.
Chloral hydrate	Weak	There is no evidence of PPARα activation by chloral hydrate aside from it being a weak peroxisome proliferator.

[a]A strong weight of evidence is defined as evidence from several studies which support the mode of action, while a weak weight of evidence is defined as having a single study from a single laboratory or a significant amount of contradiction in the literature (Klaunig et al. 2003).
Abbreviations: DCA, dichloroacetic acid; TCA, trichloroacetic acid; TCE, trichloroethylene.

FINDINGS AND RECOMMEDATIONS

Hepatotoxicity

The existing data clearly demonstrate that trichloroethylene produces hepatotoxicity in experimental animals and humans that is dependent on generation of reactive intermediates by CYP-450 in the liver. Besides its hepatotoxic potential, trichloroethylene and its metabolites produce liver effects categorized as independent of hepatotoxicity. These effects include elevations in plasma bile acids and accumulation of liver glycogen in the absence of subclinical evidence of liver dysfunction. The absence of liver dysfunction in rodents has been documented in studies using serum markers of liver injury and is further supported by histopathologic examinations that showed no ultrastructural changes. However, the absence of liver dysfunction in humans has been based entirely on measures of serum markers of liver injury (e.g., plasma transaminases, bilirubin concentrations). Therefore, the possibility that humans might have discrete ultrastructural changes in the liver that can affect bile acid homeostasis cannot be ruled

TABLE 4-12 PPARα Mode-of-Action Dose-Response Relationships

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
Causal Events							
PPARα Activation							
	TCA 1.0 mM; 5 mM	In vitro	DMSO	24 hr	N/A	Cos-1 cells transfected with human and mouse PPARα	Maloney and Waxman 1999
	DCA, 1.0 mM, 5 mM	In vitro	DMSO	24 hr	N/A	Cos-1 cells transfected with human and mouse PPARα	Maloney and Waxman 1999
	TCA, 4 mM (DCA at 4 mM, no effect)	In vitro	Unknown	24 hr	N/A	HL8.5 cells transfected with mouse PPARα	Walgren et al. 2000b
Regulation of Growth Regulatory Genes							
c-*jun*, c-*myc*	TCE 1000 mg/kg	Oral gavage, 5 days/wk	Corn oil	33 days	Female	$B6C3F_1$ mice	Tao et al. 1999
c-*jun*, c-*myc* (in tumors)	DCA, 20 mmol/L	Drinking water	Water	46 wk	Female	$B6C3F_1$ mice	Tao et al. 1999
c-*jun*, c-*myc* (in tumors)	TCA 20 mmol/L	Drinking water	Water	46 wk	Female	$B6C3F_1$ mice	Tao et al. 1999
c-*jun*, c-*myc*	TCE, 1000 mg/kg	Oral gavage	Corn oil	5 days	Female	$B6C3F_1$ mice	Tao et al. 2000b
c-*jun*, c-*myc*	TCA, 500 mg/kg	Oral gavage	Water	5 days	Female	$B6C3F_1$ mice	Tao et al. 2000b
c-*jun*, c-*myc*	DCA, 500 mg/kg	Oral gavage	Water	5 days	Female	$B6C3F_1$ mice	Tao et al. 2000b
Growth regulatory genes via microarray	TCE, 1500 mg/kg	Oral gavage	Methyl cellulose (0.1%)	3 days	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
Cell Proliferation or Apoptosis							
BrdU labeling	TCE, 500 and 1,000 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
[^{3}H]Thymidine incorporation	TCE, 500 mg/kg	Oral gavage	Corn oil	7, 14 days	Male	$B6C3F_1$ mice	Klaunig et al. 1991

TABLE 4-12 *Continued*

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
[^{3}H]Thymidine incorporation	TCE, no effect	Oral gavage	Corn oil	3, 7, 14 days	Female	B6C3F$_1$ mice	Klaunig et al. 1991
[^{3}H]Thymidine incorporation	TCE, no effect	Oral gavage	Corn oil	3, 7, 14 days	Male	F344 rat	Klaunig et al. 1991
[^{3}H]Thymidine incorporation	TCE, no effect	Oral gavage	Corn oil	3, 7, 14 days	Male	F344 rat	Klaunig et al. 1991
BrdU labeling (not within tumors)	DCA, 2 g/L	Drinking water	Water	14 days	Male	B6C3F$_1$ mice	Stauber and Bull 1997
BrdU labeling (not within tumors)	TCA, 2 g/L	Drinking water	Water	14, 28 days	Male	B6C3F$_1$ mice	Stauber and Bull 1997
[^{3}H]Thymidine incorporation	TCE, 250-2,500 mg/kg	Intraperitoneal	Corn Oil	24 hr (500, 1,250) 36 hr (250-1,250) 48 hr (all doses) 72 hr (250, 1,250, 2,500) 96 hr (none)	Male	Sprague-Dawley rats	Soni et al. 1998
Anchorage-independent growth	TCA, DCA, 0.5-2 mM	In vitro	Media	10-25 days	Male	Hepatocytes from B6C3F$_1$ mice	Stauber et al. 1998
[^{3}H]Thymidine incorporation	TCA, DCA 0.1-1 mM. Varied based on individual	In vitro	Media	72 hr	Male and Female	Human primary cultures	Walgren et al. 2000a
PCNA labeling	TCA, DCA 500 mg/kg	Oral gavage	Saline	72-96 hr	Female	B6C3F$_1$ mice	Ge et al. 2001
Clonal Expansion[b]							
Associative Events							
Peroxisome Proliferation and Regulation of Lipid Metabolism Genes							
CYP 4a12 mRNA	TCE, 1,500 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 days	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
CYP4A, ACO protein,	TCE, 125, 500, 1,000 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004

TABLE 4-12 *Continued*

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
Palmitoyl CoA oxidase	TCE, 1,500 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
CYP4A, ACO protein,	TCA, 1.0, 2.0 M (not observed in PPARα null)	Drinking water	Water	1 week	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
Palmitoyl CoA oxidase	TCA, 2 M (not observed in PPARα null)	Drinking water	Water	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
CYP4A protein	DCA, 2 M (not observed in PPARα null)	Drinking water	Water	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
Palmitoyl CoA oxidase	TCA, DCA 600 mg/kg	In vitro	Media	24 hr	Male and Female	$B6C3F_1$ mouse liver homogenate and primary culture. No effects seen with LEH rat or human cells/cultures	Walgren et al. 2000a
Laural CoA oxidase	DCA, 3.2 g/l Unaffected by methionine	Drinking water	Water	8 and 44 wk	Female	$B6C3F_1$ mice	Pereira et al. 2004
Lauric acid oxidase; CYP4A protein	CH, 100 mg/kg	Gavage (5 days/week)	Water	104 wk	Male	$B6C3F_1$/Nctr mice	Leakey et al. 2003a
Palmitoyl CoA oxidase activity	TCA, 5 g/L MCA, no effect	Drinking water	Water	15-104 wk	Male	F344 rats	DeAngelo et al. 1997
Palmitoyl CoA oxidase activity	Perc, 200, 400 ppm	Inhalation	Air	28 days	Male and Female	F344 rats $B6CF_1$ mice	Odum et al. 1988
Peroxisome number	Perc, 200, 400 ppm	Inhalation	Air	28 days	Male and Female	$B6CF_1$ mice	Odum et al. 1988
Palmitoyl CoA oxidase activity	MCA, 500 μM DCA, 1000 μM TCA, 500 μM	In vitro	Media	72 hr	Male	Long Evans rat primary hepatocytes	Walgren et al. 2004
Peroxisome proliferation	TCE, 0.75 g/kg	Gavage (daily)	Corn oil	2 wk	Male and Female	Sv/129 (not seen in PPARα null)	Nakajima et al. 2000

TABLE 4-12 *Continued*

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
Peroxisome proliferation	TCE, 0.75 g/kg	Gavage (daily)	Corn oil	2 wk	Male	Sv/129 (not seen in PPARα null or in female wt or null)	Nakajima et al. 2000
Palmitoyl CoA oxidase activity	DCA, 0.5 mM (mouse); 1.0 mM (rat)	In vitro	Media	72 hr	Male	$B6C3F_1$ mice, Long Evans rat primary hepatocytes	Everhart et al. 1998
Palmitoyl CoA oxidase activity	TCE, 100 mg/kg TCA, 500 mg/kg Perc, 1,000 mg/kg (¼- ½ the effect seen with Wy14,643 at 50 mg/kg)	Gavage (10 days)	DMSO/corn oil	10 days	Male	$B6C3F_1$ mice, F344 rats	Goldsworthy and Popp 1987
Oxidative stress[c]							

[a]Doses where statistically significant effects were observed.
[b]Clonal expansion is pertinent only in reference to tumor formation and phenotyping of foci and nodules.
[c]Signs of oxidative stress, including glycogen accumulation.
Abbreviations: BrdU, bromodeoxyuridine; CH, chloral hydrate; DCA, dichloroacetic acid; DMSO, dimethyl sulfoxide; MCA, monochloroacetic acid; Perc, tetrachloroethylene; TCA, trichloroacetic acid; TCE, trichloroethylene.

out. There are some mechanistic data addressing the nature of the elevation in bile acids in plasma but the precise mode of action remains unknown.

Elevation of plasma bile acids could result in their accumulation in other tissues, which could conceivably have detrimental effects on those organs. In addition to their lipid-solubilizing effect, bile acids are also signaling molecules that regulate gene transcription. The farnesoid X receptor functions as a bile acid nuclear receptor which regulates transcription of multiple genes responsible for maintaining cholesterol and bile acid homeostasis. Thus, accumulation of bile acids might contribute to the adverse effects of trichloroethylene exposure in organs other than the liver through a detergent effect or altered cellular signaling. However, it is not clear whether or not this is a significant effect.

The human relevance of liver glycogen accumulation observed in rodents exposed to dichloroacetic acid remains unclear. There are no studies documenting this effect in humans. Furthermore, all research on glycogen accumulation has been carried out using dichloroacetic acid, and not trichloroethylene. In light of this, it is not known whether exposure to trichloroethylene at environmentally relevant concentrations results in glycogen accumulation in rodents or humans.

Investigators have been able to dissociate the glycogen deposition effect from the peroxisome proliferation produced by trichloroethylene and its metabolites because dichloroacetic acid, which produces significant liver enlargement and no peroxisome proliferation, induces a marked accumulation of liver glycogen. In contrast, exposure to trichloroacetic acid produces only modest glycogen accumulation while stimulating considerable peroxisome proliferation.

Data from studies with autoimmune-prone mice also suggest that trichloroethylene and its metabolites are capable of triggering an immune-mediated reaction against the liver. This observation is highly relevant to humans because there are multiple case reports of workers exposed to trichloroethylene with Stevens-Johnson syndrome who developed generalized skin reactions often accompanied by hepatitis of acute onset.

In summary, data generated since EPA (2001b) released its draft health risk assessment have not significantly advanced understanding of whether some of the noncancer liver effects of trichloroethylene and its metabolites are independent of early ultrastructural and discrete pathologic changes. Also, the relationship of these effects to hepatocarcinogenesis remains unclear.

Liver Cancer

Data on trichloroethylene indicate that relatively high doses are needed to induce liver cancer, even in susceptible strains of mice. The three major metabolites of trichloroethylene—trichloroacetic acid, dichloroacetic acid, and chloral hydrate—can contribute to liver cancer in mice. None of the three is directly mutagenic or genotoxic as the principal mode of action. Trichloroacetic acid and dichloroacetic acid have been shown to promote liver cancer in classic initiation-promotion experimental protocols. The concentrations of trichloroacetic acid in blood required to induce liver cancer approach the millimolar range, whereas dichloroacetic acid concentrations in blood associated with carcinogenesis are in the submicromolar range. The carcinogenic activity of chloral hydrate is largely dependent on its conversion to trichloroacetic

acid and dichloroacetic acid. Dichloroacetic acid and trichloroacetic acid adequately account for the hepatocarcinogenic responses to trichloroethylene.

There is sufficient weight of evidence to conclude that the mode of action of trichloroacetic acid as a rodent liver carcinogen is principally as a liver peroxisome proliferator in a specific strain of mouse, B6C3F_1. This strain also has a particularly high background incidence of liver tumors. Moreover, F344 rats in which peroxisome proliferation is not induced do not show induction of liver cancer at the same doses at which B6C3F_1 mice do. Altered cellular metabolism leading to transient changes in cell proliferation and cell regulation is related to induction of peroxisome proliferation in rodents.

Dichloroacetic acid produces liver tumors with a different phenotype than trichloroacetic acid. Its tumorigenic effects are closely associated with differential effects on cell replication rates in tumors, normal hepatocytes, and suppression of apoptosis. There is sufficient weight of evidence to conclude that the mode of action of dichloroacetic acid at high doses in rodents includes hepatomegaly and marked cytomegaly, which are closely associated with its activity as a differential promoter with effects on increased cell replication rates in tumors and normal hepatocytes and suppression of apoptosis. High-dose treatments alter activities of key enzymes in metabolism and cell growth. Dichloroacetic acid induces liver tumorigenesis in both mice and rats by this mode of action. However, dichloroacetic acid is a minor metabolite of trichloroethylene and whether it is formed in humans has not been clearly established.

The mode of action of chloral hydrate as a weak rodent liver carcinogen is dominated by induction of peroxisome proliferation activity in male B6C3F_1 mice. Female mice and rats are resistant to the carcinogenic effects of chloral hydrate. Because the metabolites of chloral hydrate are trichloroacetic acid and dichloroacetic acid, the contribution to liver tumor induction of the specific modes of action of each of these metabolites is also likely; however, an overall lack of potency for chloral hydrate in the carcinogenic response is notable.

Induction of peroxisome proliferation in human liver is not a prominent feature; therefore, this key event related to trichloroacetic acid liver carcinogenesis is not likely to occur in humans. The promotional activity of dichloroacetic acid includes a significant effect on cellular metabolism, differentiation function, and proliferation that encompass a mitogenic mode of action. Repeated exposure to dichloroacetic acid results in an inhibition of both mitosis and apoptosis and eventual formation of focal eosinophilic hyperplastic lesions. Assuming that the underlying mode of action for dichloroacetic acid as a liver carcinogen in rodents is promotional events affecting and culminating in mitogenesis, genotypic species differences between mice (one transforming growth factor type-β growth factor receptor allele) and humans with two functional copies of the gene suggest that humans would be phenotypically much less susceptible to liver carcinogenesis from agents that demonstrate a mitogenic mode of action (Andersen et al. 1995).

The weak carcinogenic activity of chloral hydrate in the liver of male B6C3F_1 mice (with no liver cancer induction in female mice and rats) combined with lower rates of oxidation and higher rates of conjugation in humans compared with mice indicates that the mode of action in mice is not likely to be relevant to humans.

Exposure to trichloroethylene at concentrations relevant to the general public is not likely to induce liver cancer in humans. However, it is possible that much higher exposures to trichloroethylene, such as in certain high-risk occupations or in heavily contaminated locales, could result in increased risks of liver toxicity and cancer. In addition, the existence of sensitive populations due to genetics, disease, or life stage cannot be discounted.

Recommendations:

- Additional laboratory studies are needed to establish the significance of increased bile acids in relation to the hepatotoxic potential of trichloroethylene, as well as in relation to other systemic effects. Such studies will help clarify whether elevation of serum bile acids is an early indicator of changes in liver function or is a marker of exposure to trichloroethylene (or other halogenated solvents which induce this effect).
- More research is also needed to assess whether increases in serum bile acids in humans exposed to trichloroethylene are independent of discrete pathologic changes in the liver. Because histopathologic assessment would be difficult to perform in human subjects, new, highly sensitive, and noninvasive toxicologic parameters are needed to clarify the toxicologic importance of these effects of trichloroethylene.
- Additional studies of the effects of trichloroethylene on glycogen accumulation, perhaps using cultured human hepatocytes, should shed some light on the significance of this effect and its relevance to humans.
- More research is needed to determine whether an autoimmune response might play a role in trichloroethylene-mediated liver disease. Adducts formed between metabolites of trichloroethylene and liver proteins can result in the formation of neoantigens. These neoantigens can lead to antibody-dependent hepatocellular injury. The same process has been reported with chemicals such as halothane, which is well known to produce immune-mediated hepatotoxicity. Studies similar to those carried out with halothane could be instrumental in elucidating whether autoimmunity is a causal factor in the hepatotoxicity of trichloroethylene.
- Studies are needed to determine the metabolic pathway and yield for forming dichloroacetic acid from trichloroethylene either via trichloroacetic acid or via other pathway(s). If dichloroacetic acid is found to be a metabolite of concern, additional studies may be needed to understand its role in the toxicities associated with trichloroethylene.

The epidemiologic evidence for an association between liver cancer and trichloroethylene exposure is inconclusive. Excess liver cancer incidence was observed in most of the cohort studies that examined this outcome. However, cohort studies of mortality and population-based case-control studies yielded mixed results. Of particular interest is a recent case-control study that found an association between liver cancer mortality and trichloroethylene concentrations in well water in a community that was downstream from a Taiwanese factory. Although this study suffers from several methodologic weaknesses, it is the first to show an association between environmental exposures to trichloroethylene and liver cancer mortality.

5

Reproductive and Developmental Toxicity

This chapter discusses essential scientific issues about the reproductive and developmental toxicity of trichloroethylene, focusing on the issues of hazard characterization and mode of action for trichloroethylene toxicity. The chapter assesses the available information from animal, in vitro, and human studies. First, evidence for reproductive toxicity from laboratory studies is discussed. Second, developmental toxicity studies of trichloroethylene in different species are discussed, followed by information from in vitro studies that are relevant to assessing the mode of action for certain effects. Third, the evidence is considered in humans for reproductive and developmental effects together, as many epidemiologic studies evaluated reproductive and development outcomes together.

ANIMAL STUDIES OF REPRODUCTIVE TOXICITY

Studies of trichloroethylene on male reproductive end points have been done primarily in rodents. Zenick et al. (1984) reported that trichloroethylene at an oral dose of 1,000 mg/kg/day (5 days/week for 6 weeks) inhibited copulatory behavior in male Sprague-Dawley rats. Because the effects occurred during the first few weeks of exposure and returned to normal after 5 weeks, the narcotic properties of trichloroethylene were suspected to be responsible for the initial changes in copulatory behavior. No effects were observed on semen plug weights or on sperm counts, motility, or morphology (Zenick et al. 1984).

A study of male mice exposed to trichloroethylene via inhalation found significantly increased percentages of abnormal sperm at the highest test concentration (approximately150 parts per million [ppm]). Because the mice were exposed during early spermatogenesis and not for a full spermatogenic cycle, the authors concluded that the observed spermatotoxicity occurred during the first or second meiosis or during sperm maturation (spermiogenesis) (Land et al. 1981).

Forkert et al. (2002) used an inhibitory CYP2E1 monoclonal antibody to demonstrate that the enzyme CYP2E1 is involved in trichloroethylene metabolism to chloral in both the testes and epididymides. The extent of chloral formation was higher in the epididymis than in the testis and correlated with the relative levels of CYP2E1 activity present in individual tissues. Exposed

mice exhibited damage to the epididymal epithelium, which plays a central role in sperm development and functional maturity during sperm transit from the testis to the cauda epithelium. Because CYP2E1 is localized in the male reproductive tract of mice, monkeys, and humans and trichloroethylene and its P-450-derived metabolites are found in the seminal fluid of exposed humans and in the epididymides of exposed mice (Forkert et al. 2003), the effects on sperm development and functional maturity in rodents are likely to be predictive of outcomes in humans.

DuTeaux et al. (2002) proposed that the epididymis might be toxicologically analogous to the kidney. They found that enzymes involved in nephrotoxic responses (e.g., CYP2E1, soluble epoxide hydrolase, and the cysteine conjugate β-lyase) are present in the epididymis and efferent ducts of rats. The enzymes were present at higher concentrations in the efferent ducts than in the epididymis, suggesting that the proximal excurrent ducts are a potential target for compounds and metabolites that are nephrotoxicants, such as trichloroethylene.

Kumar et al. (2000a,b) also found significant decreases in total epididymal sperm count, motility, and specific activities of the steroidogenic enzymes glucose-6-phosphate dehydrogenase and 17β-hydroxysteroid dehydrogenase, with concomitant decreases in sperm testosterone, in male Wistar rats exposed by inhalation to trichloroethylene at 376 ppm (4 hours/day, 5 days/week, for 12 or 24 weeks). Fertility was reportedly reduced when the males mated with unexposed females (Kumar et al. 2000b). Follow-up investigations of whether testicular steroid precursors (cholesterol and ascorbic acid) or testosterone plays a role in trichloroethylene-induced effects showed that total cholesterol content was greater in the testes of rats exposed to trichloroethylene than in controls (Kumar et al. 2000b). The authors concluded that the findings indicated possible impairment of testicular testosterone biosynthesis, which might explain, at least in part, the reproductive inefficiency initially reported.

Another study by Kumar et al. (2001b) explored the histomorphology of the testes, sperm count and motility, and marker testicular enzymes involved in sperm maturation and spermatogenesis. They found significant reductions in body and testis weights, total cauda epididymal sperm count, and percent motile sperm after 12 and 24 weeks of exposure. Histologically, at 12 weeks, the testes exhibited fewer spermatogenic cells and spermatids in the seminiferous tubules, with some of the spermatogenic cells appearing necrotic. After 24 weeks, the testes were atrophied and harder, with smaller seminiferous tubules. Leydig cells were hyperplastic. All the tubules had Sertoli cells but were almost devoid of spermatocytes and spermatids. "Fibrines" were present in the tubular lumens. Testicular dehydrogenase and glucose-6-phosphate dehydrogenase were significantly reduced, and glutamyltransferase and β-glucuronidase were significantly increased.

The authors concluded that postmeiotic stages of spermatogenesis in rats were susceptible to trichloroethylene-induced insult. They suggested that exposure to trichloroethylene "may cause testicular toxicity, which in turn affects postmeiotic cells of spermatogenesis, Sertoli cells, and Leydig cell functions" (Kumar et al. 2001b). Whether the reproductive changes were transient or permanent was not assessed, nor were epididymal sperm counts or motility reported. It is also not clear whether the three studies reported are three separate studies, as the same design and the same trichloroethylene concentrations were used.

Xu et al. (2004) evaluated the effects of trichloroethylene on CD-1 male mice exposed by inhalation to trichloroethylene at 1,000 ppm for 6 hours/day, 5 days/week, for 1-6 weeks. There were no effects on body, testis, or epididymal weights nor on the number or percent motility of epididymal plus vas deferens sperm. More than 95% of sperm retrieved from trichloroethylene-

exposed and control mice exhibited normal morphology. In vitro incubation of sperm from trichloroethylene-exposed males with eggs from superovulated unexposed females resulted in significant decreases in the number of sperm bound per egg when the males were exposed for 2 and 6 weeks (but not after exposure of 1 or 4 weeks). In vivo fertilization with exposed males and superovulated females resulted in significant reductions in the percentage of eggs fertilized after 2 and 6 weeks of exposure to trichloroethylene (the slight reduction after 4 weeks was not statistically significant). The sperm-egg binding assay (with sperm exposed in vitro) indicated significant decreases in the number of sperm per egg when sperm were pretreated with chloral hydrate (0.1-10 μg/mL) and decreased, but to a lesser extent, with trichloroethanol treatment (0.1-10 μg/mL). The authors concluded that exposure to trichloroethylene leads to impairment of sperm's fertilizing ability, which may be attributed to the trichloroethylene metabolites chloral hydrate and trichloroethanol (Xu et al. 2004).

DuTeaux et al. (2003, 2004) investigated the bioactivation of trichloroethylene and adduct formation in the rat epididymis and efferent ducts and whether trichloroethylene caused oxidative damage to sperm. They reported that the cysteine conjugate β-lyase, which bioactivates the trichloroethylene metabolite dichlorovinyl cysteine to a reactive intermediate, was localized in the efferent ducts and epididymis (the soluble but not the mitochondrial form). Both forms of β-lyase were detected in the kidney. When rats were dosed with dichlorovinyl cysteine, no protein adducts were present in the epididymis or efferent ducts, but adducts were detected in the renal proximal tubules. Trichloroethylene can also be metabolized (and form protein adducts) through the cytochrome P-450-mediated pathway. Immunoreactive CYP2E1 was localized to the efferent ducts and corpus epididymal epithelia. Trichloroethylene metabolism was inhibited 77% when efferent duct microsomes were preincubated with an antibody to CYP2E1. Dichloroacetyl adducts were detected in epididymal and efferent duct microsomes exposed to trichloroethylene in vitro. The authors suggested that CYP2E1-dependent metabolism of trichloroethylene to reactive metabolites and the subsequent covalent binding to cellular proteins may be involved in the male reproductive toxicity of trichloroethylene (DuTeaux et al. 2003).

DuTeaux et al. (2004) conducted an in vivo study in male Sprague-Dawley rats exposed to trichloroethylene in drinking water at 0.2% or 0.4% (v/v) for 14 days. There were no treatment-related changes in testes and epididymides weight, sperm concentration, or sperm motility. Flow cytometry indicated no treatment-related differences in sperm mitochondrial potential or acrosomal stability. Trichloroethylene caused slight histologic changes in efferent ductal epithelium, coinciding with ductal localization of CYP2E1. There were no alterations in the testis or in any segment of the epididymis, but the rats exhibited significant dose-related reductions in percent fertilized ova from untreated females in vitro. However, the magnitude of the latter effect differed in rats from different sources, with rats from Charles River Laboratories having a greater percentage of fertilized oocytes than rats from a breeding colony at the University of California at Davis. Because there were no changes in sperm indices and no pathologic lesions to explain the reduced fertility, the authors used immunochemical techniques to detect oxidized sperm protein. The tests showed "halos" of oxidized proteins around the sperm head and midpiece from trichloroethylene-treated males. Dose-dependent increases in lipid peroxidation were observed in sperm from trichloroethylene-treated males as well. The authors suggested that oxidative damage to sperm may explain the reduced fertilizing capacity in trichloroethylene-exposed males and "provide another mechanism by which [trichloroethylene] can adversely affect reproductive capabilities in the male" (DuTeaux et al. 2004).

Veeramachaneni et al. (2001) reported that a mixture of drinking water pollutants, including trichloroethylene, caused alterations in mating desire and ability, sperm quality, and Leydig cell function in rabbits. Although there is no way to parse out the contribution, if any, from trichloroethylene on these rather subtle and subjectively assessed effects, this is the only paper on trichloroethylene that used the rabbit, which is considered the most sensitive species for detection of male reproductive toxicity.

In a study of B6D2F_1 pregnant mice, no effect of trichloroethylene was observed on maternal, reproductive, or offspring parameters at an oral dose of 140 mg/kg/day during gestation (Cosby and Dukelow 1992). The authors also performed in vitro studies of mouse eggs cultured with cauda epididymal sperm and trichloroethylene or its metabolites—dichloroacetic acid, trichloroacetic acid, and trichloroethanol—to assess the effects on fertilization. No effect on fertilization was found with trichloroethylene at concentrations up to 1,000 ppm. Dichloroacetic acid and trichloroacetic acid each showed a dose-related decrease in the percent of eggs fertilized that was significant at 1,000 and 100 mg/kg, respectively. Trichloroacetic acid and trichloroethanol individually and in combination also exhibited significant reductions in the percentage of fertilized embryos, but the combination did not exhibit a synergistic effect (Cosby and Dukelow 1992).

NTP conducted reproductive-assessment-by-continuous-breeding studies of trichloroethylene in CD-1 mice (NTP 1986a) and Fischer 344 rats (NTP 1986b). Both species received feed containing microencapsulated trichloroethylene at 0.15%, 0.30%, and 0.60% (w/w). In mice, the most significant finding was perinatal mortality (61% in the high-dose group versus 28% in the controls). Sperm motility was reduced by approximately 45% in F_0 males and by 18% in F_1 males. No effects on mating, fertility, or reproductive performance were found (NTP 1986a).

In the Fischer 344 rats, a monotonic trend was present for fewer litters per mating pair (from 3.5 in controls to 2 in the high-dose group; the middle- and high-dose groups had 9% and 16% fewer pups per litter, respectively). Crossover mating indicated reduced mating (75%) in groups with a treated parent. Number of pups per litter, viability, and weight of the pups were not affected. There were no changes in sperm indices. Reduced male body weights, reduced absolute testis weights, and increased adjusted seminal vesicle weights were found at necropsy. The conclusion was that trichloroethylene produced some general toxicity (reduced body weight gains, and increased relative liver and kidney weights) at all doses, whereas reduced reproductive indices were observed only in the F_1 rats at the middle and high doses (NTP 1986b).

Manson et al. (1984) investigated reproductive performance in female Long-Evans hooded rats exposed to trichloroethylene by gavage at doses of 10, 100, or 1,000 mg/kg/day (in corn oil). The rats were dosed two weeks prior to mating, during the one-week mating period (5 days per week), and from gestational day 0 to 21 (7 days per week). Trichloroethylene and its major metabolites, trichloroacetic acid and trichloroethanol, were measured in the female reproductive organs and neonatal tissues. At the end of the premating period, trichloroethylene concentrations were uniformly high in fat, adrenal glands, and ovaries across groups, while uterine tissue had relatively high concentrations of trichloroacetic acid. Female fertility was unaffected. Four of 23 females treated with trichloroethylene at 1,000 mg/kg/day died (and one had a fully absorbed litter), and weight gain was significantly reduced throughout the treatment period. Neonatal survival was significantly reduced at 1,000 mg/kg/day, with the majority of deaths among female offspring at the time of birth. Trichloroacetic acid concentrations in blood, liver, and milk contents of the stomach of female (but not male) neonates exhibited a dose-

related increase across groups. The authors concluded that oral exposure to trichloroethylene at doses below those that cause maternal toxicity did not affect fertility or pregnancy outcome and that the accumulation of trichloroethylene and trichloroacetic acid in ovaries, adrenal glands, and uteri had no impact on mating success. No information on any structural anomalies was provided. The lack of any effects on offspring survival argues against any functional consequences, except for the preferential loss of offspring females at birth, which remains unexplained.

Berger and Horner (2003) exposed female rats to a number of male reproductive toxicants, including trichloroethylene and tetrachloroethylene, and assessed the fertilization of their oocytes by sperm from unexposed males in vitro. Female Simonson albino rats were administered drinking water containing trichloroethylene at 0.45% for 2 weeks. They were induced to ovulate, and their oocytes were incubated with semen from unexposed male rats from the same colony. Trichloroethylene significantly reduced fertilizability of the oocytes (46% versus 57% in the vehicle control females [$P < 0.005$]). Trichloroethylene also significantly reduced the number of penetrated sperm per oocyte (0.70 per oocyte versus 0.81 per oocyte in the vehicle control [$P < 0.05$]). Oocytes from trichloroethylene-exposed females also had reduced ability to bind sperm plasma membrane proteins compared with oocytes from the vehicle controls ($P < 0.05$).

Tetrachloroethylene, administered in drinking water at 0.9%, reduced the percentage of females ovulating compared with the vehicle values (53% versus 78%, $P < 0.05$). There were no effects on oocyte fertilizability or on the number of penetrated sperm per oocyte. Nose-only inhalation exposure of females to tetrachloroethylene at 1,700 ppm for two 1-hour periods per day for 2 weeks slightly reduced the fertilizability of oocytes (from 85% to 80%, $P < 0.05$). The number of penetrated sperm per oocyte was more obviously reduced (1.6 for exposed females versus 2.5 for unexposed females). There were no clinical signs of toxicity. Berger and Horner (2003) view their work as the first documented in vivo effect on oocyte fertilizability by a reproductive toxicant for a female mammal (for trichloroethylene and tetrachloroethylene). Because there is evidence that both compounds are male reproductive toxicants via a mechanism that does not involve the endocrine system, these results in females support their hypothesis.

ANIMAL STUDIES OF DEVELOPMENTAL TOXICITY

Avian and Mammalian Species

The avian explant model is used for descriptions and mechanistic studies of heart development and teratogenesis (as well as for other organ system development) because of the conservation of developmental stages and perturbations across vertebrates, especially birds and mammals, and because of the access to, and visibility of, developing avian structures in ovo and in vitro. Studies of trichloroethylene in the in ovo development of chicks have reported increased mortality and developmental defects, including lighter pigmentation, edema, evisceration (failure of abdominal wall closure, gastroschisis), decreased growth, beak malformations, club foot, and patchy feathering (Bross et al. 1983). Loeber et al. (1988) reported cardiac defects that involved inflow and outflow abnormalities, including septal defects, conotruncal abnormalities, atrioventricular canal defects, hypoplastic ventricle, and abnormalities in cardiac muscle.

Dorfmueller et al. (1979) compared the effects of timing of exposure to trichloroethylene on reproductive outcomes of female Long-Evans hooded rats exposed to trichloroethylene by inhalation at concentrations of 1,800 ± 200 ppm. Groups of rats were exposed before mating only, during pregnancy only, and throughout pre-mating, mating, and pregnancy. There were no effects of any exposure paradigm on maternal body or liver weights or on pre- or post-implantation loss, litter size (live, dead, resorbed, total), resorption rate, fetal body weight, or sex ratio. Fetal skeletal anomalies (predominantly incomplete ossification of sternum) and soft tissue anomalies (displaced right ovary) were significantly increased only in the group exposed during gestation. The investigators considered these effects to be evidence of developmental delay in maturation rather than teratogenesis. Variable effects were observed in the mixed function oxidase enzyme assay which did not correlate with treatment or pregnancy state. However, when the two groups with and the two groups without gestational exposure were compared, a significant increase in ethoxycoumarin dealkylase was associated with gestational exposure. Behavioral evaluation of the pups indicated no effect of treatment in general motor activity in any groups at any age. A reduction in postnatal body weights was observed in the offspring of mothers with pregestational exposure. The authors concluded that "No results indicative of treatment-related maternal toxicity, embryotoxicity, serum teratogenicity, or significant behavioral deficits were observed in any of the treatments groups" (Dorfmueller et al. 1979, p. 153).

Schwetz et al. (1975) exposed timed-pregnant Sprague-Dawley rats and Swiss Webster mice to trichloroethylene by inhalation at a concentration of 300 ppm (twice the maximum allowable excursion limit for human industrial exposure defined by the American Conference of Governmental Industrial Hygienists) for 7 hours/day on gestational days 6-15. No effects from trichloroethylene were found in rat or mouse dams (except for a statistically significant 4-5% reduction in maternal body weights in rats) or conceptuses using standard Segment II developmental toxicity assessments, including pre- and post-implantation loss, litter size, fetal body weight, crown-rump length, and external, visceral, skeletal, and total malformations and variations.

Smith et al. (1989, 1992) studied the trichloroethylene metabolites trichloroacetic acid and dichloroacetic acid in pregnant Long-Evans rats and found that both metabolites reduced body weight and growth and produced cardiac defects. The most common findings after treatment with trichloroacetic acid were levocardia (at 330 mg/kg/day and greater) and interventricular septal defect (800 mg/kg/day and greater). With dichloroacetic acid, resorptions significantly increased at 900 mg/kg/day; the most common cardiac malformations were a defect between the ascending aorta and right ventricle (at 140 mg/kg/day and greater), levocardia (at 900 mg/kg/day and greater), and intraventricular septal defect (at 1,400 mg/kg/day and greater). Thus, trichloroacetic acid appears to be more potent than dichloroacetic acid in causing cardiac teratogenicity, although both compounds exhibited dose-response relationships. The authors did not find a no-observed-adverse-effect level for trichloroacetic acid, but they concluded that the no-observed-adverse-effect level for the developmental toxicity of dichloroacetic acid in rats was 14 mg/kg/day (Smith et al. 1992).

A follow-up series of four studies on dichloroacetic acid were performed to determine the most sensitive period of development and to further characterize the heart defects (Epstein et al. 1992). The heart defects found were predominantly high interventricular septal defects and, less commonly, interventricular septal defects. The authors suggested that high interventricular septal defects are a specific type of defect produced by a failure of proliferating interventricular

septal tissue to fuse with the right tubercle of the atrioventricular cushion tissue. In the proposed model, of the three foramina (primum, secundum, and tertium) initially present, a single interventricular foramen is eventually obliterated. They also proposed that dichloroacetic acid interferes with closure of the interventricular foramen tertium, allowing the aorta to retain its embryonic connection to the right ventricle. In these studies, disruption of these septation processes did not affect the aortic connection with the left ventricle. The authors rightly questioned why dichloroacetic acid has a selective effect on fetal cardiogenesis. They speculated that perhaps the dichloroacetic acid target is a unique cell type at a unique time—the biochemical differentiation of cardiocytes (Epstein et al. 1992).

Dawson et al. (1990) reported that continuous delivery of trichloroethylene into the gravid uteri of Sprague-Dawley rats resulted in increased incidence of fetal heart malformations (on a fetal basis—that is, number of fetuses with heart defect[s]/number of fetuses examined). The incidence was 9% with trichloroethylene at 15 ppm and 14% with trichloroethylene at 1,500 ppm, compared with a 3% incidence in the control group (an approximately 36% increased incidence at a 100-fold increase in exposure).

In another study with a more conventional experimental design, Dawson et al. (1993) exposed Sprague-Dawley rats to trichloroethylene (1.5 or 1,100 ppm) or dichloroethylene (0.15 or 110 ppm) in drinking water before pregnancy, during pregnancy, and both before and during pregnancy. They found no differences among groups in the percentage of live births, uterine implants, or resorptions. There were also no differences among groups in congenital abnormalities other than cardiac defects. However, it is unclear how completely teratogenesis was evaluated. Of the 238 fetuses in the control group, 3% had cardiac defects (2.5% of the more than 600 fetuses in control groups in this and previous studies exhibited cardiac defects). In this study, the high concentrations of trichloroethylene and dichloroethylene were 733% higher than the low concentrations, but the increased incidence of cardiac malformations was only 13% with trichloroethylene and 12% with dichloroethylene. The dose-response curve was extraordinarily flat.

To evaluate the proximate teratogen(s) responsible for the fetal cardiac malformations associated with trichloroethylene and dichloroethylene, Johnson et al. (1998a,b) tested several metabolites of the two compounds in Sprague-Dawley rats. They found an increased incidence of cardiac malformations with trichloroacetic acid at a concentration of 2,730 ppm (10.53% versus 2.15% in the cumulative control group; $P = 0.0001$ for fetuses and $P = 0.0004$ for affected litters). The cardiac malformations included atrial septal defect, perimembranous ventricular septal defect, pulmonary artery hypoplasia, aortic hypoplasia, mitral valve defect, muscular ventricular septal defect, and pulmonary valve defect. Increased cardiac defects were not found with the other metabolites tested (monochloroacetic acid, trichloroethanol, trichloroacetaldehyde, dichloroacetaldehyde, carboxymethylcysteine, and dichlorovinyl cysteine). The metabolite dichloroacetic acid was not evaluated in this study. The investigators asserted that the low number of cardiac defects found in the metabolite groups (other than trichloroacetic acid) does not preclude teratogenicity, because the study might not have had enough statistical power to detect an effect. They also asserted that the study does not prove that trichloroacetic acid is a human cardiac teratogen. Limitations associated with the study include discrepancies in the number of affected hearts and fetuses reported in the paper and failure to disclose that the control group was not concurrent.

Johnson et al. (2003) sought to identify a threshold dose of trichloroethylene in rats. They reclassified the data reported by Dawson et al. (1993) and assessed them with information

on two lower test concentrations (0.0025 and 0.25 ppm). The authors concluded that their analysis identified "a threshold level of less than [0.25 ppm trichloroethylene] above which rats exposed to increasing levels of [trichloroethylene] during pregnancy have increasing incidences of cardiac malformations in their fetuses."

Fisher et al. (2001) also evaluated trichloroethylene (500 mg/kg/day) and the metabolites trichloroacetic acid (300 mg/kg/day) and dichloroacetic acid (300 mg/kg/day) for teratogenicity. The two metabolites produced significantly reduced fetal body weights on both a per fetus and a litter basis. They found no statistically significant increases in the incidence of fetal heart malformations by litter or fetus for trichloroethylene or the two metabolites. The incidences were 4.5%, 3.3%, and 4.7% for trichloroethylene, trichloroacetic acid, and dichloroacetic acid, respectively. Interestingly, the rate of cardiac malformations observed in the treatment groups, although not different from the concurrent controls, was similar to those reported in the treatment groups by Johnson et al. (1998a,b) and Dawson et al. (1993). Of note, the frequency of the abnormalities in the soybean oil control group were higher in this study (6.5%) than in the control groups (individually and grouped) of Johnson et al. (1998a,b, 2003) and Dawson et al. (1993). Such a difference would decrease the power to detect a difference.

Collier et al. (2003) studied the effects of trichloroethylene, dichloroethylene, and trichloroacetic acid on gene expression in rats during cardiac development. They found up-regulated transcripts including genes associated with stress response (*Hsp70*) and homeostasis (several ribosomal proteins). Down-regulated transcripts included extracellular matrix compounds (GPI-p137 and vimentin) and Ca^{2+} responsive proteins (Serca-2 Ca^{2+}-ATPase and β-catenin). Down-regulated sequences appear to be associated with cellular housekeeping, cell adhesion, and developmental processes. Two possible markers for fetal trichloroethylene exposure were Serca-2 Ca^{2+}-ATPase and GPI-p137.

Collier et al. (2003) considered that cardiac insufficiency is a "plausible explanation" for the reduced incidence of reported malformations from in utero exposure to trichloroethylene. They argued that a lack of exposure studies in rats and mice terminated before embryonic day 18 (mice) or day 21 (rats) would exclude findings of gross cardiac defects inconsistent with life that could be identified only early in gestation (these conceptuses would die before term). The authors therefore associated "the limited reports of cardiac defects associated with [trichloroethylene] exposure with timing of the analysis, not an absence of cardiac-related effects from exposure" (Collier et al. 2003, p. 495). However, the timing of necropsy and fetal heart examinations in rodent models has been the same for researchers reporting rodent fetal heart malformations and researchers not reporting those effects. Consistent with the changes in gene regulation that Collier et al. observed, trichloroethylene and trichloroacetic acid, but not trichloroethanol or chloral, inhibited in vitro gap-junction-mediated intercellular communication, an important part of cellular adhesion and cardiac development (Klaunig et al. 1989).

Coberly et al. (1992) used the mouse embryo chimera assay to evaluate the effects of trichloroethylene on preimplantation embryos. Superovulated female CD-1 (Swiss) mice were treated with trichloroethylene intraperitoneally (0, 0.01, 0.02, or 10 μg/kg) or by gavage (0, 0.1, and 1.0 μg/kg; 0, 48.3, and 483 mg/kg) when the embryos were traversing the pronuclei stages of development. Embryos were flushed from excised oviducts and scored for numbers, embryonic stages, and viability for each female. The stages included degenerate and 1-cell embryos, and 2- and 4-cell embryos. All 4-cell embryos from females within a dose group were pooled, and the chimeras constructed from them. No treatment-related effects were seen on the total number of

embryos recovered from the oviducts of trichloroethylene-treated females, and no significant cell proliferation decreases were observed for any of the experimental chimeric embryos.

Other Species

Relevant toxicity studies have been performed in animal models other than rodent and avian species, including daphnids and amphibians. Niederlehner et al. (1998) evaluated the reproductive response of the daphnid *Ceriodaphnia dubia* to industrial chemicals alone and as mixtures of trichloroethylene, benzene, toluene, ethylbenzene, *m*-xylene, and tetrachloroethylene. The reproductive median inhibition concentration was 82 μM for trichloroethylene and 4 μM for tetrachloroethylene. Mixtures of trichloroethylene, benzene, and toluene had effects at concentrations below their individual lowest-observed-effect levels. In addition, observed responses to mixtures differed significantly from that predicted from a concentration-addition model, with the predicted relationship overestimating mixture toxicity (Niederlehner et al. 1998).

The Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) has been used to study the developmental toxicity of trichloroethylene. Trichloroethylene has tested positive in this assay (Fort et al. 1991, 1993). The trichloroethylene metabolites dichloroacetic acid, trichloroacetic acid, trichloroethanol, and oxalic acid have also tested positive in the FETAX assay, but each was significantly less toxic than trichloroethylene. It was suggested that trichloroethylene oxide, a highly embryotoxic epoxide intermediate formed from mixed-function oxidation-mediated metabolism, might play a significant role in the developmental toxicity of trichloroethylene (Fort et al. 1993).

Embryonic larvae of four North American amphibian species—wood frogs (*Rana sylvatica*), green frogs (*Rana clamitans*), American toads (*Bufo americanus*), and spotted salamanders (*Ambystoma maculatron*)—were exposed to tetrachloroethylene and its metabolites, trichloroethylene and *cis*- and *trans*-dichloroethylene. Tetrachloroethylene and trichloroethylene were teratogenic to amphibian embryos, with median effective concentrations (EC_{50}s) (malformations) of 12 mg/L for tetrachloroethylene in wood frogs and 40 mg/L for trichloroethylene in green frogs; these concentrations did not affect embryonic survival. American toads were less sensitive, with no EC_{50} for developmental abnormalities attained at the highest test concentrations (tetrachloroethylene at 45 mg/L and trichloroethylene at 85 mg/L) (McDaniel et al. 2004).

In Vitro Studies

Saillenfait et al. (1995) used rat whole embryo cultures to retain embryonic structural integrity and to preclude the presence of maternal absorption, distribution, metabolism, and excretion. They exposed explanted Sprague-Dawley embryos (gestational day 10) to trichloroethylene, tetrachloroethylene (with or without microsomes), or one of four chlorinated compounds (trichloroacetic acid, dichloroacetic acid, chloral hydrate, and trichloroacetyl chloride). They found concentration-dependent decreases in growth and differentiation indices and increases in the incidence of morphologically abnormal embryos with all the test chemicals. Trichloroethylene and tetrachloroethylene produced qualitatively similar patterns of

abnormalities, whereas their metabolites produced distinguishable dysmorphic profiles. The presence of hepatic microsomal fractions in the culture medium enhanced embryotoxic effects. Embryo lethality was defined as loss of heartbeat; the percentage of explants with a heartbeat ranged from 36% with trichloroethylene and 43% with tetrachloroethylene to 86% and 100%, respectively, in the presence of the microsomal biotransformation system. Heart defects were not mentioned. All treatments at higher doses caused a treatment-related reduction in the first branchial arch, and an abnormal brain was the most prominent effect noted. Incomplete closure of the neural tube was also noted. Chloral hydrate caused pericardial dilation (at 2 mM, with 100% embryo lethality at 2.5 mM). With respect to embryo lethality, the order of potency for metabolites was chloral, trichloroacetyl chloride, dichloroacetic acid. The dose-response curve for embryo lethality was steep. Trichloroethylene at 15 mM caused malformations but no embryo deaths, but 30 mM was 90% embryo lethal. Tetrachloroethylene caused 10% embryo lethality at 7.5 mM and 83.5% embryo lethality at 15 mM.

A number of in vitro studies of the effects of trichloroethylene and its metabolites on cardiac valve formation have been performed. The basic events of cardiac valve formation in mammals (including humans and laboratory animals) and birds are as follows:

1. Early in development (in utero or in ovo), the heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothelial cell layer. Between the two cell layers is extracellular matrix.
2. At a specific time in development, a subpopulation of endothelial cells lining the atrioventricular canal detaches from adjacent cells and invades the underlying extracellular matrix (Markwald et al. 1984). This event is termed an epithelial-mesenchymal cell transformation, when at least three distinct events occur: endothelial cell activation (chick stage 14), mesenchymal cell formation (chick stage 16), and mesenchymal cell invasion (migration) into the extracellular matrix (chick stages 17 and 18) (Boyer et al. 2000a).
3. Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and begin proliferating to populate the entire atrioventricular canal extracellular matrix.
4. The cardiac mesenchyme provides the cellular constituents for the septum intermedium and the valvular leaflets of the mitral (bicuspid) and tricuspid atrioventricular valves. The septum intermedium subsequently contributes to the lower portion of the interatrial septum and the membranous portion of the interventricular septum (Markwald et al. 1984, 1996; Boyer et al. 2000).

The chick stage 16 atrioventricular canal can be removed from the embryo and cultured in vitro on a three-dimensional hydrated collagen gel. During the 24 to 48 hours of incubation, all the stages described above occur in vitro and can be studied with or without test chemical exposures (e.g., Mjaatvedt et al. 1987, 1991; Loeber and Runyan 1990; Ramsdell and Markwald 1997). The in vitro model has identified a number of molecules as being involved with this transformation (e.g., fibronectin, laminin, galactosyltransferase [Mjaatvedt et al. 1997]; components of the extracellular matrix [Mjaatvedt et al. 1991]; and smooth muscle α-actin and transforming growth factor β3 [Nakajima et al. 1997; Ramsdell and Markwald 1997]).

Because trichloroethylene was implicated in heart defects of the chick (Bross et al. 1983), Boyer et al. (2000) explanted chick stage 16 atrioventricular canals onto gels with medium containing trichloroethylene at 50, 100, 150, 200, or 250 ppm. The explants were evaluated for epithelial-mesenchymal transformation, endothelial cell density, and immunohistochemistry.

Atrioventricular canal explants for chick stage 17 embryos were also cultured with no chemicals. Then, medium containing 0 or 250 ppm was added for 30 minutes, and the cell migration assay was performed. Trichloroethylene affected several elements of the epithelial-mesenchymal cell transformation process, including blockage of the endothelial cell-cell separation process that is associated with endothelial activation, inhibition of mesenchymal cell formation in a dose-dependent pattern, and no effect on the cell migration rate of the fully formed mesenchymal cells. The expression of three proteins selected as molecular markers of the epithelial-mesenchymal transformation was analyzed. Trichloroethylene inhibited the expression of transcription factor Mox-1 and extracellular matrix protein fibrillin 2 but had no effect on expression of smooth muscle α-actin. The authors suggested that trichloroethylene might cause cardiac valvular and septal malformations by inhibiting early endothelial separation and early events of mesenchymal cell formation in the embryonic heart (Boyer et al. 2000). Another interpretation (Hoffman et al. 2004) is that trichloroethylene affects the adhesive properties of endocardial cells. On the other hand, other have questioned the relevance of this study based on concerns that the concentrations used would not be tolerated by animals or achieved in humans (Dugard 2000). No direct experimental data are available that address trichloroethylene concentrations present in cardiac tissue in vivo.

Hoffman et al. (2004) proposed the using a whole embryo explant culture as a better system to evaluate the effects on the formation of the valves and septa of the heart, as anatomic relationships among tissues and organs are maintained and embryonic development can be monitored over the course of the experiment. Because mesenchymal cells first appear in the atrioventricular canal extracellular matrix at chick stage 16, they explanted stage-14 embryos for culture with trichloroethylene concentrations of 0, 10, 40, or 80 ppm. Only comparably staged and morphologically identical control and trichloroethylene-treated embryos were analyzed further by scanning laser confocal microscopy to assess cellular characteristics of the endocardial cushion tissues. With a trichloroethylene concentration of 80 ppm, there was a reduction (58.3% of the control value) in total cells of the atrioventricular cushion and an altered distribution of mesenchymal cells within the cushion. (Embryos treated with 40 ppm trichloroethylene were not assessed.) The authors also tested trichloroacetic acid in their whole embryo explant systems, and it too altered the distribution of cells in the endocardial cushions (Hoffman et al. 2004).

Using an in vitro mouse conceptus model in which haloacetic acids were added individually to culture medium, Hunter et al. (1996) showed that haloacetic acids generally are capable of causing altered development of the neural tube, eye and pharyngeal arches, and heart. With the exception of higher (≥250 μM) concentrations of monochloracetic acid, no increased embryo death was seen. Trichloroacetic acid was not teratogenic at 1,000 μM. At 2,000 μM, increased neural tube defects and fewer somites were observed. At 3,000 μM, an increase in eye, pharyngeal arch, and heart defects was seen. The cardiac anomalies were predominantly incomplete looping; a reduction in cardiac length beyond the bulboventricular fold and a reduction in the caliber of the heart tube lumen also were observed. Dichloroacetic acid was not teratogenic at 734 μM, but a significant, albeit inconsistent, decrease in somite number was variably observed at 1,468 μM or greater. Increased neural tube defects occurred at 5,871 μM, whereas pharyngeal defects and cardiac defects were observed at concentrations of 7,339 μM or greater. Extremely high concentrations (11,010 μM) caused rotational, eye, and somite dysmorphology. Virtually all haloacetic acids produced neural tube defects, but the potency varied by four orders of magnitude. The authors calculated benchmark concentrations for neural tube defects (defined as the lower 95% confidence interval of the concentration of acid required

to produce a 5% increase in the number of embryos with neural tube defects) of 91, 1,336, and 2,452 μM for monochloroacetic acid, trichloroacetic acid, and dichloroacetic acid, respectively. Generally, the chloroacetic acids were less potent than the bromoacetic acids but more potent than the fluoroacetic acids (Richard and Hunter 1996). This evidence that multiple halogenated compounds might be teratogenic supports the need for studies of outcomes after combined exposures.

Direct extrapolation of the results of direct embryo culture studies is limited because maternal absorption, excretion, and metabolism do not occur in in vitro systems. In addition, no conceptuses were exposed simultaneously to multiple haloacetic acids, all of which are frequently low-level water disinfection products, or to other common coexposure chemicals, including compounds metabolically upstream and downstream of the haloacetic acids, such as trichloroethylene and chloral hydrate. However, such models allow intrinsic toxicity to be evaluated.

Finally, one in vitro assay used bovine coronary endothelial cells cultured in medium containing 10% fetal bovine serum with antibiotics to suggest that endothelial nitric oxide synthase might be involved in trichloroethylene-mediated toxicity. Proliferating endothelial cells were treated with trichloroethylene at 0-100 μM and then stimulated with the calcium ionophore A23187 to determine changes in endothelial cells and endothelial nitric oxide synthase, nitric oxide, and superoxide anion generation. Trichloroethylene decreased concentrations of heat shock protein associated with endothelial nitric oxide synthase by 46.7% and inhibited vascular endothelial growth-factor-stimulated endothelial cell proliferation by 12% to 35%. These data show that trichloroethylene alters heat shock protein interactions with endothelial nitric oxide synthase and induces endothelial nitric oxide synthase to shift nitric oxide to superoxide-anion generation. The findings provide new insight into how trichloroethylene alters endothelial and endothelial nitric oxide synthase function to impair vascular endothelial growth-factor-stimulated endothelial proliferation. Such changes in endothelial function play an important role in the development of heart defects (Ou et al. 2003).

HUMAN STUDIES OF REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Currently, studies of the human reproductive and development effects of trichloroethylene consist of (1) retrospective, community-based studies of multiple pregnancy outcomes among residents of neighborhoods with varying documentation of trichloroethylene or trichloroethylene-related exposures; (2) studies of reproductive outcomes of men and women with nonquantitative occupational exposure to multiple, ill-defined organic solvents; (3) limited studies of health outcomes of children exposed to trichloroethylene, including intrauterine exposure; and (4) evaluations of spermatogenesis and sexual function among men with occupational exposure to high concentrations of trichloroethylene or trichloroethylene-related compounds. The following discussion provides a qualitative overview of the epidemiologic evidence. A more critical evaluation of relevant studies in terms of methods, exposures, and results is necessary to fully characterize the reproductive and developmental hazards of trichloroethylene (see Chapter 2 for guidance on how this should be done).

Community-Based Studies

Woburn, Massachusetts

Birth outcomes have been studied in communities of East Woburn, Massachusetts, that were served between 1964 and 1979 by wells contaminated with trichloroethylene (267 parts per billion [ppb]) and tetrachloroethylene (21 ppb). A health survey of 5,010 residents of Woburn (about 50% of the population) by Lagakos et al. (1986) found an increased likelihood of exposure to contaminated well water and ear and eye anomalies (odds ratio [OR] = 14.9; $P < 0.0001$) and perinatal deaths (OR = 10.0, $P = 0.003$) between 1970 and 1982. A combination of central nervous system, chromosomal, and oral cleft anomalies was also reported to be increased, but a review of data and the fact that this is an unconventional grouping of outcomes suggested that the finding was not plausibly related to exposure to the contaminated wells. Although no other birth defects or anomalies were reported, statistical power was limited. Spontaneous abortion and low birth weight were not increased; however, the study used a nonstandard cutoff weight to assess low birth weight (2,722 g versus 2,500 g).

A study by the Massachusetts Department of Public Health (MDPH/CDC/MHRI 1994) of the same population indicated the possibility of increased risk for small-for-gestational-age-babies in the context of exposure in the third trimester of pregnancy, particularly among teenage women (OR = 6.37; 95% confidence interval [CI] = 2.39, 16.99), and for preterm birth among older mothers with exposure in the third trimester (OR = 2.66; 95% CI = 1.14, 6.19). Others reported an interaction between maternal age and trichloroethylene (Yauck et al. 2004) and the similar compound tetrachloroethylene (Sonnenfeld et al. 2001) as well as other compounds (Fox et al. 1994; Jacobson et al. 1998). However, gestational age was not reported for more than half of the sample, making these observations unreliable.

The prevalence of structural birth defects was evaluated retrospectively between January 1975 and December 1984 and prospectively between January 1989 and March 1991. Over 4,500 hospital records were reviewed for the retrospective study, and over 11,000 for the prospective study. Ascertainment methods increased the possibility of a type II error for many birth defects, particularly congenital heart disease. The prevalence of choanal atresia (OR = 8.33, 95% CI = 2.37, 26.25; OR = 6.6, 95% CI = 1.99, 19.19) and hypospadias (OR = 1.59, 95% CI = 1.02, 2.45) was significantly higher in Woburn during the period of well contamination than in two national referent populations. Although the rates remained higher after well closure, the ascertainment methods for the post-well-closure period were more complete than during the contamination period. A referent population (such as from a retrospective analysis during the contamination years of the 12 noncontaminated communities used in the prospective study) was not included.

Camp Lejeune, North Carolina

Studies of developmental outcomes have been performed at the U.S. Marine Corps Base at Camp Lejeune, North Carolina, where drinking water was found to be contaminated with chlorinated volatile organic compounds, trichloroethylene, tetrachloroethylene, dichloroethylene, and lead. Exposure to these compounds was documented over a period of 34 months but likely occurred for years, perhaps as long as 30 years. Concentrations of trichloroethylene ranged from 8 to 1,400 ppb, dichloroethylene ranged from 12 to 407 ppb, and tetrachloroethylene ranged

from 76 to 215 ppb, depending on the water system and the time of testing. From the evaluations at Camp Lejuene to date, two potentially plausible findings appear. Trichloroethylene exposure appears to be associated with significantly smaller male infants, whether measured as a continuous variable or as a dichotomous variable (ATSDR 1998; Sonnenfeld et al. 2001). Among exposed male infants, adjusted mean birth weight was reduced by 312 g (90% CI = -540, -85; $P < 0.01$), and the prevalence of small for gestational age increased (OR = 3.9, 90% CI = 1.1, 11.9), whereas no difference was found in female infants.

Although such gender differences are not readily explained and have not been associated with trichloroethylene in other studies, male susceptibility has been seen with other chemicals, such as polychlorinated biphenyls and dioxins (Dewailly et al. 1993; Rylander et al. 1995). For tetrachloroethylene, two exposed subgroups appeared at greater risk of adverse outcomes: women over the age of 35 and those with a history of fetal loss (adjusted OR = 2.1, 90% CI = 0.9, 4.9; OR = 2.5; 90% CI = 1.5, 4.3). The adjusted differences in mean birth weight in the tetrachloroethylene-exposed infants in the two subgroups were -130 g (90% CI = -236, -23) and -104 g (90% CI = -174, -34), respectively. Increased environmental risk of birth defects among older women has been observed for trichloroethylene (Yauck et al. 2004), ethanol (Jacobson et al. 1996; Jacobson, et al. 1998), and smoking (Backe 1993; Fox et al. 1994). The association between prior fetal deaths and risk appeared to increase with the number of fetal deaths, increasing the probability that it was not a chance observation.

Limitations to the ATSDR (1998) study include the possibility of misclassification, particularly the possibility that unexposed mothers were included in the "exposed" population. This is more likely to be true in the tetrachloroethylene and "long trichloroethylene exposed" groups than in the "short trichloroethylene exposed" groups and would decrease the power to detect a difference and lead to a bias toward the null. The information about exposure for any individual is crude, as no information about water consumption was available, nor was information available about showering or other hot water activities, which would contribute to exposure by dermal and inhalation routes. Biologic monitoring information was also not available.

The clinical determination of gestational age from retrospective data is difficult and, in the ATSDR study, underestimates of gestational age likely occurred with birth weight used as a criterion because large-for-gestational-age preterm infants were removed from the study. Such an underestimate would decrease power and attenuate differences in the number of small-for-gestational-age infants between exposed and unexposed women. Removal of large-for-gestational-age preterm infants substantially decreased the number of preterm infants, which potentially decreased the power to detect a difference in prematurity rates. Data on tobacco and alcohol—other important effect modifiers—were not available. However, these exposures are less likely to have affected the exposure groups differentially.

ATSDR (2003) plans another study to assess birth defects and childhood cancer (leukemia, nonHodgkin's lymphoma) prevalence among children exposed to contaminated drinking water at Camp Lejeune. Surveys have been conducted to identify the study population and confirm the health outcomes reported by parents. A full study is planned to include all confirmed cases of birth defects and childhood cancers and an assessment of exposure to trichloroethylene and other drinking water contaminants by modeling the water system.

Santa Clara County, California

After the identification of well contamination with 1,1,1-trichloroethane, a solvent that shares some of the same principal metabolites as trichloroethylene (trichloroethanol and trichloroacetic acid), the public reported an increased number of spontaneous abortions and cases of congenital heart disease. A series of studies were done evaluating pregnancy outcomes (Deane et al. 1989; Wrensch et al. 1990a,b) and congenital heart disease (Swan et al. 1989). Deane et al. (1989) reported a higher rate of spontaneous abortions and congenital anomalies among exposed women (n = 250). The relative risk of congenital anomalies considered as a single entity was 3.1 (95 % CI = 1.1, 10.4). A later study by the same investigators (Wrensch et al. 1990a) expanded on this study and included an additional exposed area (n = 1,105). The analysis of the larger data set did not confirm the previous finding of an increase in spontaneous abortions in exposed women. An additional report (Wrensch et al. 1990b) that provided hydrogeologic assessment of the amount of exposure in two exposed census tracts found that the tract with higher concentrations of 1,1,1-trichloroethane had a lower rate of spontaneous abortions than the tract with lower 1,1,1-trichloroethane concentrations. The sample size was too small for statistical evaluation of birth defects.

The cluster of congenital heart disease in Santa Clara County was confirmed, but Swan et al. (1989) suggested that it was not likely to be related to 1,1,1-trichloroethane because the increased prevalence of congenital heart disease was not consistent across the time period when exposure occurred. However, most cases of congenital heart disease (9 of 12 cases) occurred in a region not served by the well that was the focus of the study. In fact, the cluster was closer to a well that contaminated by about 80-fold less 1,1,1-trichloroethane and smaller amounts of dichloroethylene, with perhaps slightly different time periods. The impre*cis*e assessment of exposure is such that the manuscript does not add substantial information for risk assessment.

The assessment of birth defects in the study of Wrensch et al. (1990a) included an analysis of 36 of 166 reported cases of birth defects. Only about 35% of women were interviewed for birth defect ascertainment because of out-migration. Women who remained in the area might not represent the total exposed population; those who left the area could plausibly have a higher rate of offspring with birth defects than those who remained there. A 4-fold increase in prevalence of malformations was seen in the original exposed area compared with the original unexposed area (Deane et al. 1989), but this was not replicated in the comparison of the added exposed and control areas (Wrensch et al. 1990a). In addition, the confidence intervals for the association between birth defects and exposure were wide (1.2-14.7). Also problematic is the observation that ethanol consumption during the first trimester was associated with a 2-fold lower malformation prevalence, suggesting a problem in methodology or sample size (Deane et al. 1989). Thus, the Santa Clara, California, studies are of limited value in addressing birth defects.

New Jersey

Bove et al. (1995) conducted a cross-sectional study of 80,938 births and 594 fetal deaths from 75 New Jersey towns, using records of water samples and birth and fetal death certificates for the calendar years 1985-1988. They estimated individual exposure information with information from the state monitoring program for multiple solvents. Analyses of water samples

detected trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, 1,1- and 1,2-dichloroethylene, and at least 11 other solvents at <1 ppb. Decreases in adjusted mean birth weight of greater than 20 g were seen with trichloroethylene and total dichloroethylene exposure. An association was seen between exposure to trichloroethylene and low birth weight in term infants (OR = 1.23). No association was seen with small for gestational age or prematurity. Very low birth weight was associated with tetrachloroethylene exposure greater than 10 ppb (OR = 1.49). Fetal death was marginally associated with total dichloroethylene (OR = 1.18; 50% CI = 0.9, 1.55). For central nervous system defects, they found a positive association for total dichloroethylene exposure greater than 2 ppb (OR = 2.52, 90% CI = 1.25, 5.09). Neural tube defects were associated with total exposure to dichloroethylene (OR = 2.60, 90% CI = 0.93, 6.50) and were marginally associated with exposure to trichloroethylene greater than 10 ppb (OR = 2.53, 90% CI = 0.91, 6.37). However, the relationships between central nervous system and neural tube defects and trichloroethylene exposure were not monotonic, only the continuous variable was associated. In contrast to central nervous system anomalies, the relationship between trichloroethylene and oral clefts was monotonic if concentrations greater than 5 ppb were considered (OR = 2.24, 90% CI = 1.16, 4.20). Exposure to tetrachloroethylene was also associated with oral clefts (OR = 3.54, 90% CI = 1.28, 8.78). In a model that included other similar halogens, the OR for the association between oral clefts and exposure to trichloroethylene at greater than 5 ppb increased to 3.5, whereas that of other halogenated compounds fell with trichloroethylene exposure included. No relationship was seen between trichloroethylene and major cardiac defects or ventricular septal defects. This study likely includes a substantial amount of misclassification, which would decrease the power to detect a difference and would likely attenuate associations. The definition of small for gestational age as the smallest 5% would decrease power and prohibit comparison with other studies. In addition, effect modifiers were not assessed. Importantly, the extent of testing of interactions among solvents, other than the routine inclusion of total trihalomethanes in the analyses of trichloroethylene and similar compounds, is unclear. The passive ascertainment system used would likely yield valid results for easily detectable lesions such as oral clefts, but such systems are known to miss congenital heart disease (Cronk et al. 2003). The latter would again increase a type II error.

Tucson, Arizona

Three census tracts in Tucson, Arizona, (total population 1,099) were found to have trichloroethylene-contaminated well water between 1978 and 1981. Rodenbeck et al. (2000) estimated that concentrations of trichloroethylene in water ranged from less than 5 to 107 μg/L. Individual or household exposure could not be estimated because operational data were not available, so the entire population of all three tracts was considered evenly exposed. Mean exposure estimates were not given. Birth outcomes were compared between this group and contemporaneous births in other census tracts and for births in the census tracts after the exposure period (1983-1985). An association was reported between exposure to trichloroethylene via drinking water and very low birth weight (OR = 3.3; 95% CI = 0.5, 20.6). The authors suggested a similar association in the postexposure period; however, the magnitude was even smaller and less reliable (OR = 1.68, 95% CI = 0.41, 6.8). No relationship was seen between living in the exposed tracts and low birth weight or small-for-gestational-age babies. The problem of uncertain and uneven exposure is substantial and would decrease the power to

detect a difference. In addition, it is noteworthy that the exposure in this study was likely low compared with other population studies.

An increased frequency of congenital heart disease was suspected in Tucson, Arizona, in 1973. In 1981, drinking water contaminated with trichloroethylene (up to 270 ppb [approximately 0.009 mg/kg/day for a 60 kg adult], but also dichloroethylene and chromium) was detected in eight wells in Tucson Valley. In an epidemiologic study of children born between 1969 and 1987, Goldberg et al. (1990) noted that parents of children with congenital heart disease had a 3-fold greater likelihood of work or residence contact with the trichloroethylene-contaminated water area (n = 246/707, 35%) compared with parents of two "control" populations that had exposure rates of about 10%. The study has been criticized for inappropriate control groups, imprecision in determining exposure, and inclusion of years after the wells closed. Bove et al. (2002) reevaluated the data and restricted the analysis to the years when the wells were operational. In the reanalysis, the prevalence ratio of offspring cardiac defects among first-trimester "exposed" parents compared with that of "unexposed" parents was 2.58 (95% CI = 2.0, 3.4). Bove et al. (2002) also addressed the lack of exposure interviews for a large number of Goldberg et al. (1990) cases. Assuming that the noninterviewed cases and the interviewed cases had similar exposures or alternatively that the noninterviewed cases and the general Tucson population controls had similar exposures, the prevalence of cardiac defects in the exposed areas exceeded that in the uncontaminated areas by 2.3- and 2-fold, respectively. Thus, although the study by Goldberg et al. (1990) is flawed, additional analyses of the original data by an independent group of investigators yielded similar results and suggest an association between water contamination and congenital heart disease.

Milwaukee, Wisconsin

Yauck et al. (2004) performed a case-control study of 4,025 infants to evaluate the association between maternal residence close to trichloroethylene-emitting sites and infants with congenital heart defects in Milwaukee, Wisconsin. Mothers were categorized as older (older than 38 years) versus younger, exposed versus nonexposed, and presence versus absence of congenital heart defects. The proportion of mothers who were both older and had presumed trichloroethylene exposure was more than 6-fold greater among case infants (with congenital heart defects) than among control infants (3.3% versus 0.5%). When adjusted for other variables (e.g., race, ethnicity, maternal education, smoking), the risk of congenital heart defects was more than 3-fold greater among infants of older, exposed mothers than in infants of older, unexposed mothers (adjusted OR = 3.2; 95% CI = 1.2, 8.7). Older maternal age, alcohol use, chronic hypertension, and preexisting diabetes were each associated with increased incidence of congenital heart defects, but a residence close to trichloroethylene-emitting sites alone was not. The most common congenital heart defects were muscular ventricular septal defect (26.9%), secundum atrial septal defect (22.0%), membranous ventricular septal defect (20.8%), pulmonary stenosis (19.2%), and ventricular septal defect, not otherwise specified (15.5%). Maternal age was also an independent risk factor to other adverse birth outcomes, particularly chromosomal anomalies (e.g., Down's syndrome). Removing babies with any documented chromosomal abnormalities (n = 16) from the data set did not change the results of the logistic regression analysis.

Endicott, New York

The New York State Health Department in conjunction with ATSDR began an evaluation of health outcomes among residents living in areas of Endicott, New York, where soil vapor contamination with volatile organic compounds was identified (NYDOH 2005a). In the eastern study region, trichloroethylene was the most commonly found contaminant, occurring in indoor air at 0.18 to 140 μg/m^3, whereas reported soil values in some areas exceeded 10,000 μg/m^3. In the western study area, tetrachloroethylene was the most commonly found contaminant, ranging from 0.1 to 3.5 μg/m^3. The study years included 1978 to 2002 for the outcome variables birth weight and gestational age. Congenital anomalies were identified using the New York State Congenital Malformation Registry (data from 1983 to 2000). Individual information on each birth in the study and the comparison areas was used to estimate risk for each of the outcome variables, while controlling for maternal age, race, ethnicity, education and infant gender and year of birth.

When births (n = 1,440) in both study areas were considered together, the frequency of moderately low birth weight babies increased (standardized incidence rate [SIR] = 1.65 (95% CI = 1.00, 2.58) as well as term low-birth-weight births (SIR = 2.38; 95% CI = 1.10, 4.27). This observation was attributed to elevations observed in the eastern study region, the area with the greatest trichloroethylene contamination. In analyses that adjusted for multiple demographic factors, the relative risk of poor growth in the eastern study area was greater than in the controls. The ORs were 1.44 (95% CI = 1.13-1.83) and 1.79 (1.27-2.51) for low birth weight and term low birth weight, respectively. Among the congenital anomalies evaluated, the risk for all cardiac defects, as well as the subset of major cardiac defects, was elevated when both eastern and western areas were considered (adjusted rate ratio [RR] = 1.99; 95% CI = 1.27, 3.12; and RR = 2.62, 95% CI = 1.31, 5.23, respectively). Similar significant observations were seen for these end points when the eastern area was evaluated independently. The estimates from the data for the western study area were similar.

The evaluation of health effects at Endicott is an ongoing study and additional analyses and data refinements are planned. The current study is limited by the lack of individual exposure information, including concentration and duration of exposure. Birth defect cases were not validated by record review. Insufficient power was available to evaluate most birth defects. Finally, the quality of information for gestational age, a common problem with birth certificate data, was unclear but is needed for the subsequently planned study of small-for-gestational-age births.

Occupational Studies

Male Fertility

Bardodej and Vyskocil (1956) reported decreased libido in male workers exposed to trichloroethylene, but this effect did not appear to be related to any significant decrease in urinary excretion of adrenocorticosteroids. They gave no details about the control group, so the significance cannot be assessed. Sperm counts and morphology as well as Y chromosomal nondisjunction during spermatogenesis did not differ between male factory workers exposed to trichloroethylene at least 20 hours/week and physician controls (Rasmussen et al. 1988).

Chia et al. (1996) examined the effects of exposure to trichloroethylene on spermatogenesis among electronics factory workers. Of 450 men, 85 had seminal fluid samples analyzed within 2 hours of collection for seminal fluid volume, total sperm count, sperm viability, proportion of progressively motile sperm, and proportion of normal and abnormal sperm forms. Personal monitoring of 12 workers indicated that 11 were exposed to trichloroethylene at concentrations ranging from 9 to 26 ppm and 1 was exposed at 131 ppm. The geometric mean of the overall mean 8-hour exposure was 29.6 ppm, and the mean urinary trichloroacetic acid concentration was 22.4 mg per g of creatinine. Workers were divided into "high"- and "low"-exposure groups based on whether their normalized concentrations of trichloroacetic acid in urine was greater or less than 25 mg per g of creatinine, respectively. There were no differences between groups for any of the sperm parameters including volume, motility, and morphology; the values for both groups were within the standards of the World Health Organization (WHO). However, mean sperm density (million per mL) was increased in both groups relative to WHO norms, but the low-exposure group had higher sperm density than the high-exposure group. When the sperm densities were compared with urinary trichloroacetic acid quartile levels, the incidence of hyperspermia (>120 million per mL of ejaculate) increased with increasing urinary quartiles, consistent with a dose-response relationship. Although hyperzoospermia has been implicated in infertility, the authors were cautious about drawing a link, because no additional information has been reported about trichloroethylene and hyperzoospermia (Chia et al. 1996). When they analyzed the serum endocrine profiles in the same 85 male workers, Chia et al. (1997) found that the age of workers and years of exposure to trichloroethylene were significantly negatively correlated with testosterone concentrations. Years of exposure were also significantly positively correlated with dehydroepiandrosterone sulfate concentrations and negatively correlated with sex-hormone-binding globulin concentrations. Urinary trichloroacetic acid concentrations did not correlate with any hormonal measurement. When the men were stratified by years of exposure (<3, 3-5, 5-7, and ≥7 years), follicle-stimulating hormone was significantly reduced only in men exposed >7 years. Luteinizing hormone, testosterone, and free androgen index were statistically equivalent for all durations. Sex-hormone-binding globulin was significantly reduced only for a work duration of 5-7 years, and dehydroepiandrosterone sulfate concentrations were significantly increased for 3-5, 5-7, and >7 years. The workers had no clinical abnormality in reproductive function. The authors suggested that the reduction of follicle-stimulating hormone and testosterone could be due to disruption of peripheral endocrine function via trichloroethylene-induced reduction of liver production of sex-hormone-binding globulin and that chronic exposure to trichloroethylene also might have affected adrenal function.

A third report of the same workers evaluated whether trichloroethylene affected adrenal function (Goh et al. 1998). Contemporaneous blood samples were tested for testosterone, sex-hormone-binding globulin, androstenedione, cortisol, aldosterone, and insulin. Trichloroethylene did not significantly change adrenal steroid concentrations. Sex-hormone-binding globulin was significantly reduced for 4-6 years and for >6 years of trichloroethylene exposure. Insulin was significantly reduced among those with 2-4 or 4-6 years of exposure but not among those with >6 years of exposure. The authors concluded that urinary concentrations of trichloroacetic acid were significantly correlated with serum insulin concentrations. They further stated that insulin and sex-hormone-binding globulin "responded in tandem," with the highest concentrations in workers exposed less than 2 years and significantly reduced levels of

both parameters in workers exposed more than 2 years. They also described an unrealistic triphasic duration-dependent response in insulin concentrations to trichloroethylene.

In a more recent study, Forkert et al. (2003) examined eight mechanics with clinical infertility who had occupational exposure to trichloroethylene for at least 2 years. Seminal fluid from all eight subjects contained trichloroethylene, chloral, and trichloroethanol, whereas dichloroacetic acid and trichloroacetic acid were present in only two and one sample, respectively. Neither trichloroethylene nor its metabolites was detected in the five control male seminal fluid samples. CYP2E1 was found in normal human testes and epididymides, specifically in the Leydig cells in the interstitium of the testis and the caput (head), corpus (body), and cauda (tail) of the epididymis. This finding was consistent with findings in rodents, which demonstrated that CYP2E1 was involved in trichloroethylene metabolism in these tissues (see earlier discussion of studies on rodents and nonhuman primates, including those by Forkert et al. 2002, 2003).

Pregnancy Outcomes

McMartin et al. (1998) performed a meta-analysis of five retrospective studies evaluating pregnancy outcome after maternal exposure to organic solvents (Eskenazi et al. 1988; Lipscomb et al. 1991; Windham et al. 1991). Sample sizes ranged from 570 to 2,950 to yield a total of 7,036 pregnancies. They selected studies for inclusion if the outcomes included spontaneous abortion before 20 weeks, and they were either case-control or cohort studies that involved first-trimester maternal inhalational exposure to organic solvents in an occupational setting. The results indicated a small, equivocally significant effect of occupational exposure on spontaneous abortion (summary OR = 1.25, 95% CI = 0.99, 1.58). The summary OR was higher (1.54; 95% CI = 1.07, 2.21) when an unpublished study of the solvent styrene was removed from the analysis. Results of the subanalysis of the cohort studies and the case-control studies were fairly similar, as were the results when unpublished studies were excluded. However, the implications for trichloroethylene per se are impossible to know, as the definition of solvent was very broad and ill-defined.

In a later prospective study, 125 pregnant women occupationally exposed to solvents were compared with 125 unexposed women matched for age, gravidity, smoking, and alcohol histories (Khattak et al. 1999). Significantly more malformations occurred among fetuses of exposed than unexposed pregnant women (RR = 13, 95% CI = 1.9, 99.5). Major malformations were more common among women who prospectively reported temporally associated exposure symptoms (eye and respiratory irritation) than among occupationally exposed, but asymptomatic, women (12/75 versus 0/43; $P < 0.001$). No pattern of association was detected in this small study of a mixture compounds. Exposed women were more likely to have had previous miscarriages, but the rates of malformations were similar among exposed women who did and did not have histories of miscarriage.

Taskinen et al. (1989) conducted a case-control study nested in an occupational cohort who were monitored biologically for exposure to six organic solvents, including tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane. Biological samples (n = 13,132) were obtained from approximately 6,000 men. Spontaneous abortion was associated with increased paternal exposure to solvents in general (adjusted OR = 2.3, 95% CI = 1.1, 5.0). They observed no association between paternal halogenated hydrocarbon exposure as a class or

with exposure to tetrachloroethylene, trichloroethylene, or 1,1,1-trichloroethane. However, the total number of fathers exposed to any halogenated hydrocarbon was 92 (31 cases and 61 referents). The sample size was too small to test associations between organic solvents and birth defects.

In another study of women who were biologically monitored (8,547 samples from 3,265 women) for exposure to solvents, Lindbohm et al. (1990) found that exposure to solvents was more common among women who had spontaneous abortions than in controls (adjusted OR = 2.2, 95% CI = 1.2, 4.1). The sample size for assessing individual solvents was small; 42 subjects were exposed to halogenated hydrocarbons (n = 14 cases, 28 controls). The ORs for associations between spontaneous abortion and exposure to individual solvents were 1.4 (95% CI = 0.5, 4.2) for tetrachloroethylene, 0.6 (95% CI = 0.2, 2.3) for trichloroethylene, and 3.4 (95% CI = 0.7, 16.9) for 1,1,1-trichloroethane. No clearly significant associations were seen for any of these solvents.

In a prospective study of 3,216 pregnant women, no association was seen between exposure to organic solvents and having a small-for-gestational-age offspring (Seidler et al. 1999). However, the exposure assessment used a relatively crude exposure tool, the Pannett job exposure matrix, and exposure to organic solvents was low. No women reported high exposure; only 23 and 73 women reported moderate and low exposure, respectively. In addition, selection bias occurred; recruitment methods decreased the likelihood that higher-risk pregnancies were included.

Using a multicenter European case-control study with six congenital malformation registries between 1989 and 1992, Lorente et al. (2000) examined occupational exposures of women (100 mothers of babies with oral clefts and 751 mothers of healthy babies) who worked during their first trimester of pregnancy. After adjustment for potential confounding factors (such as center of recruitment, maternal age, urbanization, socioeconomic status, and country of origin), only cleft palate was significantly associated with maternal occupation in services such as hair dressing (OR = 5.1, 95% CI = 1.0, 26.0) or housekeeping (OR = 2.8, 95% CI = 1.1, 7.2). The analyses further suggested that several occupational exposures were associated with orofacial clefts. Cleft palate as an isolated anomaly was associated with trichloroethylene exposure (OR = 6.7, 95% CI = 0.9, 49.7). Furthermore, among patients with cleft palate only, the risk increased with the concentration and frequency of trichloroethylene exposure. For low exposure to trichloroethylene, the OR was 6.6 (95% CI = 0.6, 79); for medium exposure it was 13.9 (95% CI = 1.1, 186). Because oral clefts are among the most frequent congenital anomalies (with a prevalence in Europe of 1 in 700 births) and are multifactorial in origin, and because this study involved a limited number of subjects, the results should be interpreted with caution.

Shaw et al. (1992) reported the findings of a case-control study of congenital heart disease among Santa Clara County births during the calendar years 1981-1983. Mothers of cases (n = 141) were more likely to report occupations associated with organic solvent exposures than mothers of controls (OR = 1.8, 95% CI = 0.95, 3.3). Problems with this study include heterogeneity of cardiac defects, the likelihood of recall bias, the exclusion of some lesions, and, importantly, the fact that exposure to contaminated drinking water was not included, even though it was known that county residents were exposed to trichloroethylene-contaminated water during part of the study period.

In the large Baltimore-Washington study of congenital heart disease, exposure to degreasing solvents (such as trichloroethylene), was 8 and 12 times more likely among mothers of infants with left-sided flow-obstructive lesions and aortic stenosis, respectively (Loffredo et

al. 1991). Other, less robust, human epidemiologic studies have evaluated occupations likely to involve exposure to solvents. In these studies, mothers of infants with congenital heart disease were roughly twice as likely as controls to have exposures to organic solvents (McDonald et al. 1987; Tikkanen and Heinonen 1988).

FINDINGS AND RECOMMENDATIONS

The fundamental question for this chapter is whether there is necessary and sufficient evidence from the animal and epidemiologic studies that trichloroethylene, at environmentally relevant doses or concentrations, causes adverse effects on reproduction or birth outcomes. In synthesizing the large body of literature addressing developmental and reproductive toxicity, the committee identified those end points for which the animal and human evidence generates the greatest level of plausibility. These end points are discussed below and include impaired intrauterine growth, cardiac teratogenesis, and altered spermatogenesis. Although the evidence suggests that trichloroethylene can generate such effects, the lowest-observed-adverse-effect level for human risk assessment remains unclear. Some information suggests that certain human subpopulations might be at increased risk because age, genetic polymorphisms, or disease (see Chapter 9). Selection of these three end points indicates not that other reproductive or developmental end points do not have an association with trichloroethylene, but rather that the combined human and animal evidence generated to date does not reach levels of reasonable plausibility.

Intrauterine Growth

The collective data on the developmental toxicity of trichloroethylene provide substantial evidence that trichloroethylene in drinking water might cause impaired intrauterine growth at environmentally relevant concentrations. Substantial decreases in fetal growth were found among offspring of women who lived in areas of Camp Lejuene, North Carolina, with contaminated water systems (ATSDR 1998). Plausibility is increased by the observation that longer exposure was associated with a marked decrease in birth weight. This observation is replicated in an exposed population in New Jersey (Bove et al. 1995), albeit with a smaller, though statistically significant, diminution in birth weight. Furthermore, a statistical decrease in birth weight was seen among offspring whose mothers were exposed to tetrachloroethylene (Sonnenfeld et al. 2001). A recent ATSDR report found decreased intrauterine growth in mothers who lived in areas with trichloroethylene and tetrachloroethylene contamination (NY Department of Health, ATSDR report). In addition, the association between increased risk of poor fetal growth among older mothers exposed to tetrachloroethylene is similar to that of other solvents, such as ethanol, increasing the plausibility of this observation. In animal studies, decreased intrauterine growth after maternal trichloroethylene exposure has been found consistently (Bross et al. 1983; Smith et al. 1989, 1992; Johnson et al. 1998a,b; Fisher et al. 2001). However, in rodent studies, dichloroacetic acid at doses as low as 140 mg/kg/day was associated with this effect (Smith et al. 1992).

Recommendation: Additional studies to delineate subpopulations at greatest risk as well as to determine the mechanisms for the putative gender and maternal age-based susceptibility are warranted. Such interactions might be confirmed with analysis of existing epidemiologic data sets.

Cardiac Teratogenicity

Cardiac teratogenicity is the developmental end point in animal studies that has received the greatest attention. The committee is aware that considerable controversy has existed regarding cardiac teratogenesis, with some reviewers on both sides of the argument (Kaneko et al. 1997; Johnson et al. 1998b; Bove et al. 2002; Hardin et al. 2005). Multiple studies in several animal models, including mammalian (Smith et al. 1989, 1992; Epstein et al. 1992; Dawson et al. 1993; Drake et al. 2006) and avian (Bross et al. 1983; Loeber et al. 1988), suggest that trichloroethylene, or one or more of its metabolites (trichloroacetic acid and dichloroacetic acid), can cause cardiac teratogenesis. Of the studies performed, the avian studies are the most convincing, and mechanistic studies in birds have been performed. Although some rodent studies have shown effects (Smith et al. 1989, 1992; Dawson et al. 1993; Epstein et al. 1992), other studies have not (NTP 1985, 1986b; Fisher et al. 2001), suggesting either methodological or strain differences. The committee noted that the rodent studies showing trichloroethylene-induced cardiac teratogenesis at low doses were performed by investigators from a single institution. Also noted were the unusually flat dose-response curves in the low-dose studies from these investigators. For example, the incidences of heart malformations at trichloroethylene concentrations of 1.5 and 1,100 ppm (almost three orders of magnitude greater) were 8.2% to 9.2% (prepregnancy and during pregnancy) to 10.4% (during pregnancy only) (Dawson et al. 1993). The same pattern occurred with dichloroethylene. Thus, the animal data are inconsistent, and the apparent species differences have not been addressed.

Of the human epidemiologic studies, the Bove et al. (2002) reanalysis of the widely criticized, but positive, study by Goldberg et al. (1990) also found a positive association. Methodological problems limited the committee's consideration of the Santa Clara County data for congenital heart disease. The recent report of an increased incidence among residents of the Endicott, New York, area was also consistent with the Goldberg study. Of note, the effect size of a 2- to 3-fold increase in risk is similar across multiple studies. Plausibility for trichloroethylene-induced cardiac teratogenesis is increased by the fact that the most frequently observed cardiac defects in the human studies, those of the interventricular septae and the valves, are consistent with the most common defects seen in the animal studies. In addition, these specific defects are consistent with mechanistic studies demonstrating altered increased proliferation in the endocardial cushions at low dose (Drake et al. 2006) or alterations in endothelial cell activation and decreased expression of the transcription factor Mox-1 and extracellular matrix protein fibrillin 2, two markers of epithelial mesenchymal cell transformation, a key process in valve and septum formation (Boyer et al. 2000). Evidence that trichloroacetic acid and dichloroacetic acid are as potent as the parent compound suggests that CYP2E1 metabolic activation, as well as the fractional formation of trichloroacetic acid from chloral, is important in trichloroethylene cardiac teratogenesis.

Recommendations: Additional studies evaluating a lowest-observed-adverse-effect level and mode of action for trichloroethylene-induced developmental effects are needed to determine the most appropriate species for human modeling. More information is needed on metabolic activation in the avian model to evaluate interspecies differences, tissue-specific concentrations of trichloroethylene and its metabolites, and human data with better ascertainment of congenital heart disease and improved quantitative assessment of trichloroethylene exposures. Reanalysis, or perhaps additional data collection, from previous epidemiologic studies could be performed. For example, for some studies, more appropriate control data might be derived, which would cost-effectively improve the assessment of human trichloroethylene teratogenesis. The interaction of trichloroethylene with other solvents, some of which are known teratogens (e.g., ethanol and toluene), might also be pursued.

Reproductive Toxicity

On the basis of evidence generated by multiple authors in multiple rodent studies (Land et al. 1981; Kumar et al. 2000a; Forkert et al. 2002), the committee suggests that trichloroethylene is toxic to spermatogenesis and sperm fertilizing ability. However, whether these effects are transient or permanent is unclear. The mode of action is unclear and might or might not relate to hormonal alterations. Critical work by Berger and Horner (2003) demonstrated that trichloroethylene and tetrachloroethylene are not only male reproductive toxicants but also female reproductive toxicants in rats. Evidence for this finding included decreased sperm penetration and decreased fertilizability of oocytes from trichloroethylene- and tetrachloroethylene-treated females and reduced sperm plasma membrane protein binding to oocytes from trichloroethylene-treated females. Metabolic activation by CYP2E1 appears necessary for toxicity; however, which of the oxidative downstream metabolites is the proximate toxicant is not yet clear. The relevance of these trichloroethylene effects on male and female reproduction in animals to adverse reproductive outcomes in humans also is not clear.

Recommendations: More research is needed to better understand the effects of trichloroethylene on sperm and oocytes and possible consequences for reproduction. Mechanistic studies are needed to determine what metabolites are responsible for the effects.

6

Neurotoxicity

This chapter comments on the discussion of neurotoxicity in the U.S. Environmental Protection Agency (EPA 2001b) draft risk assessment of trichloroethylene and reviews information on the effects of trichloroethylene on the nervous system generated since that document was released. Other recent reviews are considered, including those of the Agency for Toxic Substances and Disease Registry (ATSDR 1997a) and the New York State Department of Health (NYDOH 2005). The chapter also addresses (1) information about the effects on complex cognitive functions, (2) sensitive populations, (3) known interactions of trichloroethylene with other exposures that may affect the risk for neurotoxicity, (4) the role of trichloroethylene concentrations in the brain, (5) the potential role of trichloroethylene in the development of neurodegenerative diseases, (6) potential mechanisms of effect and their implications for complex behavioral function, and (7) research needs.

BACKGROUND

In the past, trichloroethylene was widely used as an anesthetic at concentrations of approximately 2,000 parts per million (ppm). That use was generally restricted around 1977 because of adverse effects associated with such treatments (ATSDR 1997a). Given trichloroethylene's anesthetic uses and its widespread use in occupational settings, significant information is available on the acute toxicity of trichloroethylene and its metabolites. Surprisingly, little information exists on the effects of more protracted exposures on the central nervous system, either in humans or in experimental models, particularly at lower concentrations of exposure. Where studies are available, information from human populations often relies on estimated rather than actual concentrations of exposure, making it difficult to evaluate risks to health. In addition, much of the literature related to trichloroethylene exposure in humans includes exposures to mixtures of solvents, so that it is difficult to evaluate the specific contribution of trichloroethylene to health outcomes.

ANIMAL TOXICITY

Acute Exposure

Experimental studies of acute exposures in rats have shown behavioral alterations across several functional domains at a range of concentrations that overlap with those associated with effects in humans. Most of these studies involved inhalation exposures. At higher concentrations of exposure (e.g., 1,000-4,000 ppm), reported effects include hearing loss, impaired oculomotor control, seizures, decreased wakefulness, and anesthetic effects such as lethargy and ataxia.

Auditory deficits have been observed in several studies at comparable exposure concentrations and in different strains of rats, attesting to the generality of the effects. These studies show auditory effects to occur primarily for the midfrequency tone range (Mattsson et al. 1993; Crofton and Zhao 1993; Jaspers et al. 1993; Rebert et al. 1993; Crofton et al. 1994). Studies have indicated the persistence of some adverse auditory effects, as evidenced 14 weeks postexpsoure to trichloroethylene at 4,000 ppm for 6 hr/day for 5 days (Crofton and Zhao 1993). Apparently, this outcome has not been studied after acute exposures of humans to high concentrations of trichloroethylene.

In rat models, high doses of trichloroethylene administered orally (2,500 mg/kg per day, 5 days per week for 10 weeks) result in morphologic changes in nerves, including alterations in myelination characteristics of the trigeminal nerve (Barret et al. 1991, 1992). These findings are consistent with reports of cranial nerve damage in humans (e.g., Cavanagh and Buxton 1989). A role was noted for the trichloroethylene degradation byproduct dichloroacetylene in eliciting these effects.

Effects on behavior at lower trichloroethylene concentrations in experimental studies have included impaired effortful motor response in rodents (measured by swimming performance) and decreased response of rats to avoid electric shock after a 4-hour exposure to trichloroethylene at 250 ppm (Kishi et al. 1993). The concentrations at which Kishi et al. (1993) observed effects are similar to those noted by Stewart et al. (1970) in humans reporting headaches, fatigue, and drowsiness after exposure to trichloroethylene for 7 hr/day for 5 days. ATSDR (1997a) used these studies to formulate a minimum risk level for acute duration inhalation in humans.

A newer study by Ohta et al. (2001), not available at the time of the EPA or the ATSDR review, examined the effects of trichloroethylene on long-term potentiation (an enduring increase in the efficacy of specific brain pathways), one of the hypothesized neurophysiologic mechanisms for learning. They evaluated measurements of long-term potentiation in hippocampal slices in mice 24 hours after single exposures to trichloroethylene. They observed dose-related decreases in potentiation of the action potentials of a population of neurons (population spikes) after tetanus treatment, with reductions of 15% at 300 mg/kg and of 26% at 1,000 mg/kg. The size of the area responsive to potentiation was also reduced by trichloroethylene exposure. The animals did not appear to be anesthetized by this dose. One difficulty in comparing exposures for the effects observed by Ohta et al. (2001) with those from other studies of acute exposures is the difference in route of exposure. Ohta et al. (2001) used intraperitoneal injections and did not provide any information about peak brain concentrations of trichloroethylene produced by this exposure. However, efforts should be made to estimate from physiologically based pharmacokinetic models what the peak brain concentrations would be in

this study and how they might compare with other routes for potential utility in evaluating acute-exposure risk assessment, given the nature and magnitude of the reported effect and its observation 24-hours post-exposure.

Significantly more information is available with regard to inhalational exposures in rats. Many studies have focused on sensory-based alterations in response to trichloroethylene. Reported effects include changes in the amplitude of flash-evoked potentials (visual function) (Blain et al. 1992; Albee et al. 1993), reduced acoustic startle response, and auditory-evoked potentials (auditory function) (Rebert et al. 1991; Jaspers et al. 1993), consistent with the auditory effects described above. As with higher concentrations, lower concentrations alter the shock-avoidance response (Goldberg et al. 1964).

Intermediate-Duration Exposure

Among studies using intermediate subchronic exposures, the lowest concentration of trichloroethylene associated with effects on the nervous system comes from a report by Arito et al. (1994). Rats exposed for 8 hours a day, 5 days a week for 6 weeks showed decreased wakefulness and increased slow-wave sleep during the period of exposure. When measured 22 hours after exposure, the rats showed decreased heart rates during sleep. The effects on wakefulness and sleep were observed at exposures of 50 ppm, as well as at 100 and 300 ppm, and were not dose related. Moreover, they persisted over the 6 weeks of the study (no adaptation was observed). These effects might relate to the fatigue and lethargy associated with exposure to trichloroethylene in human studies. ATSDR (1997a) used data from the 50-ppm exposure to trichloroethylene by Arito et al. (1994) to determine an intermediate-duration inhalation minimal risk level of 0.1 ppm. EPA (2001b) used a lowest-observed-adverse-effect level (LOAEL) of 50 ppm from this study to derive a pharmacokinetic-adjusted human equivalent concentration of 9 ppm and a benchmark dose associated with a 10% response (BMD_{10}) of 5 ppm. These levels of effects are directly comparable to LOAEL values determined from human studies based on chronic exposures (described later in this chapter).

Isaacson and Taylor (1989) studied the effects of trichloroethylene on rats exposed during development (gestation and lactation) at concentrations of 312, 625, and 1,250 mg/L in drinking water. These exposures were reported to increase exploratory behavior in 60- and 90-day-old offspring, with the highest exposure concentration increasing locomotor activity at 60 days of age. These exposures likewise resulted in a 40% reduction in the number of myelinated fibers in the hippocampus, a region critical to complex cognitive function (both 312 and 625 mg/L or, equivalently, 4.0 and 8.1 mg of trichloroethylene per day, respectively). It is not known, however, whether these concentrations were associated with effects on maternal weight gain during pregnancy or on litter size and pup brain and body weights. Also, doses to the pups are not known, making extrapolation difficult. Nevertheless, these effects speak to the potential for permanent damage resulting from trichloroethylene exposure during development.

Studies in gerbils reported changes in the expression of protein concentrations in the brain at lower concentrations of trichloroethylene. This includes inhalation exposures to trichloroethylene at 60 or 320 ppm for 3 months, followed by a 4-month postrecovery period, after which increases in proteins appeared in multiple brain regions, even in response to the lower concentration. DNA was elevated in two regions at 320 ppm, with a LOAEL of 60 ppm (Haglid et al. 1981). A decrease in S100 protein concentrations, thought to be a marker of brain

damage, was observed after exposure to trichloroethylene at 170 ppm or after intermittent exposure at 500 ppm for 5 months, with no postexposure recovery period (Kyrklund et al. 1984). These seemingly opposite effects of exposure to trichloroethylene could reflect the dynamics of the protein response over the period of exposure and recovery rather than discrepancies in outcome. Although these findings are potentially interesting, it is difficult to extrapolate from gerbils to humans, because kinetic characteristics of trichloroethylene in gerbils are unknown relative to rats and mice and there have been no follow-up reports in rats or mice.

Studies of subchronic exposure to trichloroethylene in rats reported after the EPA (2001b) draft risk assessment include that of Poon et al. (2002) and Oshiro et al. (2004). Poon et al. (2002) examined the effects of oral exposures to trichloroethylene at 0, 0.2, 2, 20, and 200 ppm in male and female Sprague-Dawley rats for 13 weeks. Of relevance to neurotoxicity were measures of histologic changes in the myelin sheath of the optic nerves and concentrations of biogenic amines, determined in several different brain regions in a subset of males. In the absence of reductions in food or water intake or in body weight gain, the authors reported a mild vacuolation of the myelin sheath at the highest concentration (200 ppm) in 30%-70% of the animals examined. However, there was no associated axonal degeneration or lymphoid infiltration, making interpretation of these findings difficult. It was not clear how many animals were examined for this effect, how the 30%-70% range was determined, or whether these tissues were examined in a blinded fashion. Because measurement was taken at a single time point during the exposure, it is difficult to determine the degree of damage it signifies and whether such effects were progressive with time or represent early exposure effects. No changes in biogenic amines were reported by Poon et al. (2002), as examined in frontal cortex, caudate nucleus, nucleus accumbens, hippocampus, and substantia nigra. However, the sample sizes ($n = 5$) used for this component of the study compromise the ability to detect such changes, which normally would require sample sizes up to double those used here. This problem is clear from the measures of variability presented for these data, with standard deviations as high as 50% for the control group in some cases. Thus, the general absence of effects in this study may reflect experimental parameters rather than an insensitivity of the nervous system to these concentrations of trichloroethylene. Given these limitations, the utility of these data for risk assessment is questionable despite the focus on low doses of trichloroethylene.

It appears that repeated exposures to trichloroethylene can impair sustained attention but do not seem to produce a residual impairment in this behavioral process. Bushnell and Oshiro (2000) reported that exposures to trichloroethylene at 2,000 or 2,400 ppm via inhalation for 9 days disrupted performance on a sustained attention task, decreasing the probability of a hit (correct response to a signal) and increasing response time as well as the number of response failures. Tolerance to these impairments developed over the course of exposure, however, and additional work is required to determine whether this reflected metabolic or behavioral tolerance.

In a follow-up study, Oshiro et al. (2004) examined the residual neurological effects of exposure to trichloroethylene at 0, 1,600, or 2,400 ppm for 6 hours a day for 20 days in adult male rats, with evaluation of learning a sustained attention task beginning 3 weeks postexposure. No exposure-related effects were found. Both ethanol and d-amphetamine impaired performance on the task, with a more pronounced reduction of the probability of a correct response to a signal at the highest dose of amphetamine in the group exposed to trichloroethylene at 2,400 ppm. The authors suggested that the discrepancy between the findings of this study and those of human studies that found residual deficits in cognitive function might be due to differences in the duration and number of exposures to trichloroethylene. Human studies that found residual

effects involved exposure durations ranging from a mean of 3 years to 24.5 years, whereas the exposures in the Oshiro et al. study were estimated to be equivalent to 2.6 and 3.8 years of exposure for humans. Furthermore, many previous reports of attention effects reflect exposures to mixtures of solvents rather than trichloroethylene alone, raising questions about the specific components of the exposures that would have contributed to the effects.

Although these findings suggest no residual learning deficits after trichloroethylene exposure, the differential effects of amphetamine in control versus trichloroethylene-treated animals could indicate residual effects on brain dopamine neurotransmitter systems after trichloroethylene exposure. Dopamine pathways of the central nervous system are critical for cognitive and executive functions. Moreover, they further support the potential for trichloroethylene to have protracted effects even after exposure ceases.

Waseem et al. (2001) examined neurobehavioral effects of trichloroethylene in rats after oral administration for 90 days of 350, 700, or 1,400 ppm or after inhalation exposure of 376 ppm for 4 hours per day, 5 days per week, for a total of 180 days. Locomotor activity was measured in addition to "cognition" which was evaluated using acquisition of a conditioned shock-avoidance response. Neither oral nor inhalation exposure resulted in differential effects on acquisition of the response as measured for 7 days immediately after 90 days of oral exposure or 180 days of inhalation exposure. One problem with interpretation of these studies was the experimental design in which acquisition was studied after trichloroethylene exposure. The acquisition of shock avoidance is critically dependent on the intensity of the shock stimulus. It is possible that exposure to trichloroethylene altered shock sensitivity per se. If shock sensitivity were actually reduced, one might expect a lower rate of acquisition. Thus, rates of acquisition would have to be "normalized" to shock sensitivity. Differences in sensitivity to shock per se were never compared between the two groups. In addition, rats exposed to trichloroethylene had higher levels of locomotor activity. The increased levels of motor activity also could have contributed to levels of shock avoidance, causing higher levels of movement between the chambers of the shuttle box used to measure avoidance. Although these increases were stated to be significant at days 30 and 90 of exposure and not statistically significant at day 180, the trends were still evident at 180 days, and the small number of animals used in the experiments (six per group) would likely have precluded the ability to statistically confirm such differences, particularly as locomotor activity varies substantially among animals. For these reasons, it is not possible to determine whether there were differences in learning between trichloroethylene-exposed and control animals in these experiments. Even if there were differences, the values here were not below current LOAEL values.

Chronic Exposure

Few experimental studies examining the effects of chronic exposure to trichloroethylene (>365 days) have been reported. In one study (NTP 1988), rats were administered trichloroethylene at 500 or 1,000 mg/kg per day for 103 weeks via gavage. The report described (but did not quantify) transient postdosing effects in rats that are consistent with previous reports in human and experimental exposures (lethargy, ataxia, and convulsions). One notable observation from that study suggesting a sensitization effect was that some convulsions occurred before dosing, during the weighing period. A study of mice exposed for 54 weeks to 2,400 mg/kg per day (males) or to 1,800 mg/kg per day (females) reported nonquantified observations

of excitation immediately after dosing followed by anesthetic-type effects (Henschler et al. 1984). These reports indicate a consistency of trichloroethylene's effects across species but do not provide information of particular use to the risk assessment process. Moreover, these studies examined relatively high concentrations of trichloroethylene as they were carried out in the context of carcinogenicity evaluations.

Not surprisingly, given its anesthetic properties, chronic exposures to trichloroethylene have been shown to alter neurotransmitter functions. In a study of gerbils exposed to trichloroethylene for 12 months via inhalation at 50 and 150 ppm, dose-dependent increases (52% and 97% for glutamate; 69% and 74% for γ-aminobutyric acid [GABA], respectively) were observed in the uptake of glutamate and GABA in the posterior cerebellar vermis but not in the hippocampus; perchloroethylene did not produce corresponding changes, suggesting some specificity of the effect (Briving et al. 1986). These effects were seen in the absence of any changes in body or whole brain weights and so do not appear to reflect systemic toxicity. The LOAEL for this study was 50 ppm. Difficulties in using these data include extrapolation to human exposure, given differences between the gerbil and more standard rat and mouse models for which toxicokinetic parameters are well described. Nevertheless, they appear to support the EPA-derived LOAEL of 50 ppm from Arito et al. (1994).

HUMAN TOXICITY

Acute Exposure

Effects from acute (<14 days) exposure to trichloroethylene are widely reported in humans. At lower exposures (50-300 ppm), headache, fatigue, drowsiness, and inability to concentrate are reported. As the trichloroethylene concentrations increase, dizziness, loss of facial sensation and unconsciousness can occur. With acute exposures to high concentrations (albeit highly unspecified [e.g., 1,000 ppm and above; anesthetic use was approximately 2,000 ppm]), trichloroethylene has been associated with dizziness, headache, euphoria, sleepiness, nausea, confusion, and visual and motor disturbances. Acute exposures to high concentrations, most often due to accidental occupational exposures at unspecified concentrations, have been associated with nerve damage (typically cranial nerves) and residual neurological deficits, including memory loss when measured as long as 12-18 years later (e.g., Feldman et al. 1985). A remaining uncertainty is whether the reported nerve damage results from trichloroethylene or from a metabolite.

In many reports, actual concentrations of trichloroethylene are unspecified. In a controlled exposure experiment using trichloroethylene at 100 ppm for 6 hours a day for 5 consecutive days, Triebig et al. (1977) reported no statistically significant differences between exposed and control subjects in standardized achievement tests and self-reporting scales. Stewart et al. (1970) used human volunteers exposed to trichloroethylene at defined concentrations for specified durations to evaluate changes in motor function. Outcomes in these tests were normal in response to 200 ppm, but subjects complained of fatigue and drowsiness as well as a need to exert greater mental effort on the tests, an effect that may reflect the symptoms described. In a review of the literature, ATSDR (1997a) used the study by Stewart et al. (1970) to derive an acute-duration inhalation minimum risk level. This was later adjusted to determine an intermediate-duration inhalation minimal risk level of 0.1 ppm.

Intermediate Chronic Duration Exposure

The assessment of intermediate subchronic exposures (15-364 days) reviewed by both ATSDR (1997a) and EPA (2001b) focused on studies in rats because most human studies involved chronic exposures. In general, effects from intermediate subchronic exposures are similar to those reported at higher concentrations but occur at lower trichloroethylene concentrations with more protracted duration exposures.

In a review of the published literature, ATSDR (1997a) did not cite any long-term exposure studies in humans, because exposures to trichloroethylene in these studies are unspecified (estimated rather than empirically measured). EPA (2001b) adopted a different approach, validly recognizing the adverse effects reported for humans experiencing chronic exposure. The approach reflects a weight of evidence of effects from low-dose exposures that included adverse outcomes on the central nervous system, as well as other target organs, and involved development of reference doses and reference concentrations (RfCs) and pharmacokinetic modeling. Thus, in its evaluation, EPA was more inclusive in its use of information related to longer-term exposure.

Studies of central nervous system toxicity identified by EPA include effects that are also reported in response to shorter durations and higher concentrations of trichloroethylene and, thus, represent a continuum of these effects along the dose and exposure duration-response curve, with trichloroethylene effects appearing at lower concentrations when exposure durations are longer. In addition, the LOAELs from four different animal studies examining nervous system effects show a high degree of correspondence, ranging from 20 to 50 ppm, with corresponding human equivalent concentrations of 7-16 ppm.

Among the human studies, the report by Ruijten et al. (1991) demonstrates changes in trigeminal nerve function measured using the massiter reflex latency in workers whose exposure was below the threshold limit value (35 ppm) and whose exposure duration averaged 16 years. These findings correspond to reports from studies cited above of trigeminal and cranial nerve damage at shorter-duration exposure to high concentrations of trichloroethylene. In addition, this study noted slight reductions in the sensory nerve conduction velocity and the sensory refractory period of the sural nerve, consistent with preclinical peripheral nervous system impairments and with corresponding reports from experimental studies. Because all workers had been employed as printers in the same workplace and factory, the exposures were likely highly homogenous within this group. A human equivalent concentration LOAEL of 16 ppm was determined by EPA from this report using RfC methodology for a category 3 gas, extrathoracic effects (EPA 1994b).

Rasmussen et al. (1993) examined changes in cranial nerve function, motor coordination, and vibration sensitivity in metal degreasers whose primary exposure was to trichloroethylene (determined from biomonitoring data from the Danish Labour Inspection Service). Exposure durations were shorter than in the Ruijten et al. (1991) study. Highly significant dose-related increases were seen in motor dyscoordination. These findings are impressive because they were measured by clinical neurological examination, a far less sensitive approach than is available with more sophisticated technologies (e.g., Weiss and Cory-Slechta 2001), although it is not clear whether the examiner was blind to the exposure categorization of each worker. Abnormal olfactory (cranial nerve) function was also dose related, with similar, but not significant, trends for trigeminal nerve sensory function and facial nerve function measured via taste. The human

equivalent LOAEL determined by EPA from these studies was 7 ppm using RfC methodology for a category 3 gas, extrathoracic effects (EPA 1994b).

These reports are further supported by early studies reporting symptoms of drowsiness, fatigue, headaches, and nausea in response to occupational inhalation exposures to trichloroethylene over a mean of 7-8 years, with human pharmacokinetic adjusted LOAELs using RfC methodology for a category 3 gas, extrathoracic effects (EPA 1994b), of 7-11 ppm (Okawa and Bodner 1973; Vandervort and Polakoff 1973). Moreover, all four human studies demonstrated effects in the same exposure range as the report of decreased wakefulness by Arito et al. (1994) in rats after subchronic inhalation exposures (LOAEL of 50 ppm with human pharmacokinetic adjusted value of 9 ppm and a human pharmacokinetic adjusted BMD_{10} value of 5 ppm).

Three new human chronic exposure studies have appeared since the reviews by ATSDR and EPA. Two of them examine the impact of environmental trichloroethylene exposures on neurobehavioral function (Kilburn 2002a,b). Exposures were estimated based on groundwater plumes measured during a 3-month period. Concentrations of trichloroethylene measured in well water ranged from 0.2 to 10,000 parts per billion (ppb). Exposed subjects (n = 236) lived near two electronic manufacturing plants and were involved in litigation related to these exposures; referents matched on a number of other factors (n = 161) lived in a town without contaminated water located 88 km upwind from the exposed subjects. Additional reference subjects (n = 67) were from the same geographic area as the subjects but had never lived in the exposure zone. In the first of these studies, exposed subjects were reported to have delayed simple and choice reaction times; impaired balance; delayed blink reflex latency; abnormal color discrimination; and impaired cognitive function, attention, recall, and perceptual speed (Kilburn 2002a). This study has many limitations. Exposures are estimated, not directly measured, and involve mixed solvent exposures (although the author states that the primary toxicant was trichloroethylene), examiners did not appear to have been fully blinded to treatment conditions, and the period when trichloroethylene was measured was brief. Further, subjects were involved in a lawsuit related to this exposure, introducing the potential for bias. In addition, all relevant comparisons were not made (e.g., the two reference groups were never shown to be comparable), and the pattern of differences between referents and subjects was not the same in the two groups (e.g., subjects versus local referent outcomes was not the same as subjects versus referents living 88 km distant). Thus, the reliability and utility of these findings is questionable and their relationship to exposure levels is unknown.

A second study published by the same author attempted to address the issue of the potential bias introduced by the subjects being involved in ongoing litigation (Kilburn 2002b). In this case, the 236 subjects were compared with 58 nonclaimants within the three residential areas in the exposure zone. In addition, subjects were divided into two groups based on duration of exposure (and, presumably, years of litigation as well). In addition to having the same study limitations noted above, other inconsistencies were noted. For example, subjects with shorter exposure durations to trichloroethylene had significantly abnormal sway (balance) relative to subjects with longer exposures, despite the fact that they were also 10 years younger. To examine the impact of litigation, subjects and referents were divided into three groups, each related to the area where they lived. By adopting this approach, the sample size, and thus the power to detect effects, was considerably diminished and therefore does not represent a true assessment of the impact of litigation. For example, comparisons in zone A involved 9 nonclients versus 100 clients; corresponding figures for zone B were 18 versus 16 and for zone C

were 15 versus 11. The study reports mean values but not information on variability around the mean; in all other presentations, the standard deviations were shown. Thus, these two studies do not seem adequately suited to calculate trichloroethylene risk arising from chronic exposures.

A cross-sectional study of human environmental exposure was reported by Reif et al. (2003) based on residence in a community where the drinking water had been contaminated with trichloroethylene and related chemicals between 1981 and 1986 (Rocky Mountain Arsenal Superfund site). Tests of behavior, visual contrast sensitivity, and mood were carried out for estimated exposures of ≤5, >5-10, >10-15, and >15 ppb, with 5 ppb representing the maximum contaminant level for drinking water as defined by the EPA Office of Drinking Water. Testing occurred 6 years after peak concentrations of trichloroethylene. Subjects in the study (mean ages 48.6 to 55.8 years) resided in this area for a minimum of 2 years. In this analysis, trichloroethylene at >15 ppb affected visual function (contrast sensitivity) and increased scores for confusion, depression, and tension. For behavioral function, measured using the Neurobehavioral Core Test Battery, poorer performance on the digit symbol substitution test was reported. All these effects, however, were of marginal statistical significance. Further, these studies did not directly measure exposures but were based on estimates from geographic information systems. It is also not clear to what extent the participants were aware of exposures; cleanup began in 1986. In addition, there is no information on out-migration of the population (e.g., affected individuals who may have moved from the area). The role of duration of exposure was not evaluated. Moreover, the wells were contaminated with other organic solvents, although trichloroethylene was stated to be the primary contaminant and was present at high concentrations. Collectively or individually, these limitations could increase or decrease the sensitivity of this study to detect effects. For these reasons, including these data in the trichloroethylene risk assessment should be considered cautiously.

One interesting aspect of this study, however, if reliability of the trichloroethylene exposure assessments is assumed, is the strong interactions that emerged between trichloroethylene exposure and alcohol consumption. In the group exposed to trichloroethylene at >15 ppb, the impairments in the digit span test (considered a measure of memory) were highly significant among individuals reporting alcohol use of at least one drink per month compared with no alcohol use. In addition, these individuals showed longer simple reaction time values. Deficits in memory and response time are also characteristic of higher concentration, shorter-duration exposures. Thus, alcohol appeared to exaggerate some of the behavioral effects of trichloroethylene. These findings of alcohol-trichloroethylene interactions (see also Chapter 10), while intriguing, nevertheless require caution in interpretation based on the study limitations noted above.

MODE OF ACTION

As the current literature indicates, trichloroethylene has a wide array of effects on the nervous system that may involve different mechanisms of action. For example, changes in learning and memory might be related to the impact of trichloroethylene on long-term potentiation, considered to be a neurophysiological basis of learning. Current evidence also shows that trichloroethylene affects various neurotransmitter systems. Alterations in susceptibility to chemoconvulsants after trichloroethylene exposure implicates the involvement of GABA(A) receptors (Shih et al. 2001). Studies also show effects of trichloroethylene on

serotonin neurotransmitter systems (Gerlach et al. 1998; Lopreato et al. 2003). Dopaminergic consequences (e.g., Oshiro et al. 2004) likely contribute to motor deficits associated with trichloroethylene. Mechanisms of noncarcinogenic action for other target organs may likewise be operative in the brain, including oxidative stress. As a compound that can act on peroxisome proliferator-actived receptor α (PPARα), it is important to note the existence of such receptors in brain along with their functional roles as currently established (see Appendix E for some background information on PPARα agonism).

ISSUES

Trichloroethylene and Cognitive Function

The relationship between trichloroethylene exposure parameters and impairments of complex cognitive function remain unclear. White et al. (1997) described evidence in support of impaired cognitive function. In that study, neuropsychological testing was carried out in groups of exposed individuals from three different locations (Woburn, Massachusetts; Alpha, Ohio; and St. Paul-Minneapolis, Minnesota) and percentages of each group affected on different domains (relative to normative scores) were reported. Consistently affected across all three groups were attention and executive function and memory. Many details of this study were not reported (e.g., how subjects were recruited, awareness of exposure, litigation issues), nor were individual exposure measurements available for all subjects. Although White et al. (1997) stated that cognitive deficits were more pronounced the earlier in life the exposure occurred (developmental exposures are associated with more pronounced effects), they presented no data to support that assertion. The authors also noted that effects of these environmental (oral) exposures occurred at lower concentrations than anticipated from their experience with occupational cohorts; again, comparative data were not provided. Thus, while intriguing, it is difficult to determine the significance of the findings at the current time.

Studies reported in addition to those cited above (Oshiro et al. 2001, 2004) do little to clarify the question of the parameters of trichloroethylene exposure and impaired cognition. Isaacson et al. (1990) cited improvements rather than impairments in learning, here measured using a spatial learning paradigm in young male rats exposed to trichloroethylene in drinking water. Most improved were rats that had been exposed from day 21 to day 48 and again from day 63 to day 78 of age. Estimated intake of trichloroethylene in these studies averaged 5.5 mg/day for 28 days followed by 8.5 mg/day during the second exposure interval. Similarly, an unpublished study cited by Isaacson et al. (1990) apparently observed facilitation of learning in rats exposed to trichloroethylene during development.

Many noncognitive behavioral functions can indirectly influence measurement of learning (Cory-Slechta 1989). Alterations in motor function can alter the topography or effortfulness of responding. Sensory alterations can change the discriminability of environmental signals. Alterations in motivational state can influence either the salience or the potency of a reward. All these factors must also be controlled or explored in evaluating the outcome of learning paradigms. It is not possible to determine from the experimental description whether the facilitation noted by Isaacson et al. (1990) represents a true improvement in learning, or whether it is an indirect consequence of changes in other behavioral domains (e.g., faster swim time and thus shorter delay to reward).

An example described above comes from a report by Waseem et al. (2001) that examined neurobehavioral effects of trichloroethylene in rats after oral administration for 90 days of trichloroethylene at 350, 700, or 1,400 ppm or inhalation exposure at 376 ppm for 4 hours per day, 5 days per week for a total of 180 days. The report cited a lack of effect of exposure on acquisition of a conditioned shock-avoidance response. However, this study did not control for the potential of trichloroethylene to alter levels of shock sensitivity per se, which would thereby influence the rate of acquisition of this response.

In summary, it is not yet possible to ascertain the extent of trichloroethylene-induced impairment of complex functions such as learning, memory and attention, preferential vulnerability to trichloroethylene across these domains, the exposure parameters that might be associated with any adverse effects, the extent of their reversibility, and the impact of developmental period of exposure on such effects.

Sensitive Populations

Evidence to determine the extent to which trichloroethylene exposures during development or advanced age could enhance its adverse effects on the nervous system is limited. As noted above, White et al. (1997) reported more pronounced effects of environmental trichloroethylene exposures in younger humans, but they provided no data to support these statements. Experimental studies in which the effects of developmental exposures and adult trichloroethylene exposures are directly compared have not been reported. A study by Moser et al. (1999) of oral exposure to dichloroacetic acid, a metabolite that can be formed via mixed function oxidase metabolism of trichloroethylene, does include some comparisons of weanling versus adult rats. The results presented in the paper suggest comparable effects in the end points shown, although the authors noted that neuromuscular toxicity effects appeared to be somewhat greater in rats exposed as weanlings than in those exposed as adults. Generally speaking, there is insufficient evidence to ascertain whether there are developmental differences in sensitivity to trichloroethylene-induced neurotoxicity.

Aging does appear to enhance sensitivity to the adverse effects of trichloroethylene on the nervous system. A study by Arito et al. (1994) compared the responses of 2-, 13-, 20-, and 26-month-old rats to trichloroethylene at 300 ppm for 8 hours, followed by 1,000 ppm for 8 hours, after an intervening period of clean air for 7 days. In this study, the number of incidents of spontaneous bradyarrhythmia episodes during the 28-hour period after cessation of exposure to trichloroethylene at 300 or 1,000 ppm compared with those occurring during the corresponding period of exposure to clean air was significantly greater in 20- and 26-month-old rats than in the 2- or 13-month-old rats. Measurements of trichloroethylene in brain and blood also revealed a prolonged half-life and delayed clearance with advancing age, leading the authors to posit that pharmacokinetic differences during aging may contribute to this enhanced sensitivity. Certainly, aging needs to be considered in the uncertainties associated with the risk assessment calculations.

Interactions

The extent to which trichloroethylene neurotoxicity may be altered by coexposures with other environmental or dietary constitutents is not fully elaborated. One risk modifier for neurotoxicity described by Reif et al. (2003) is alcohol. As noted above, impairments in the digit span test in response to trichloroethylene were highly significant among individuals reporting alcohol consumption of at least one drink per month, whereas no effects were observed in individuals reporting no alcohol consumption. In addition, individuals who consumed alcohol also showed longer simple reaction time values in response to trichloroethylene exposure. Thus, alcohol was noted to potentiate the effects of trichloroethylene on a measure of attention and memory. As noted previously, however, limitations of this study necessitate caution in interpreting the validity of these findings.

Significance of Brain Concentrations of Trichloroethylene

Boyes et al. (2000, 2003) explored the relationship between exposure concentration and duration under conditions of acute exposure in predicting risk of trichloroethylene neurotoxicity. The first of these studies (Boyes et al. 2000), examining hearing loss, signal detection behavior, and visual function, demonstrated that Haber's law would overestimate when extrapolating from shorter to longer trichloroethylene exposure durations and would underestimate when extrapolating from longer to shorter exposures. This study also showed that, instead, the estimated peak blood concentration of trichloroethylene at the time of testing accurately predicted the magnitude of effect on visual function and on signal detection (these neurotoxic effects reflect momentary tissue concentrations of trichloroethylene). A second study (Boyes et al. 2003) used visual evoked potentials as an outcome measure and demonstrated that the exposure metric of area under the curve was not an accurate predictor of effect; instead, brain concentration of trichloroethylene at the time of visual evoked potential testing predicted the effects of trichloroethylene across exposure concentration-duration parameters. Still questionable, however, is the extent to which this relationship would generalize to longer-term exposures.

Neurodegenerative Disease

Some reports have suggested a link between trichloroethylene exposure and Parkinson's disease. Among these are two case reports. Guehl et al. (1999) described a case of Parkinson's disease in a 47-year-old woman who had 7 years of exposure to trichloroethylene. This case is notable given the young age relative to typical onset and the fact that the subject was a woman, because time to onset is longer and Parkinson's disease incidence is lower in women than in men. Unknown, however, is the specificity of the exposure and the genetic background of the subjects. In another report, Kochen et al. (2003) cited the onset of Parkinson's disease in three individuals chronically exposed to trichloroethylene during the postexposure period. Following the case report observation, Guehl et al. (1999) also described the loss of dopamine neurons in substantia nigra pars compacta (the hallmark of Parkinson's disease) after intraperitoneal

injections of trichloroethylene at 400 mg/kg per day, 5 days a week for 4 weeks, to mice. No follow up studies to this report have been described.

Although it is not clear whether assessment of the incidence of Parkinson's disease has been examined in trichloroethylene-exposed populations, a biological basis for its potential contribution to this disease has been suggested by Riederer et al. (2002) to be based on the formation of TaClo (1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline), a potent dopaminergic neurotoxin that can be formed endogenously after exposure to the sedative chloral hydrate or after exposure to trichloroethylene. As the authors note, trichloroethylene has an estimated half-life in humans in venous blood of 21.7 hours, sufficient for the appropriate in vivo condensation reactions that would be involved in TaClo formation. Indeed, significant amounts of TaClo (approximately 200 ng per 10 mL samples) were detected in both serum and clot in Parkinson's disease patients that had been treated for several days with 500 mg of chloral hydrate (a metabolite of trichloroethylene), with blood sampled on the final day of treatment (Bringmann et al. 1999).

Additionally, there are significant structural similarities between TaClo and MPP^+ (1-methyl-4-phenylpyridinium ion), the most widely used experimental model for Parkinson's disease. As noted in the review by Riederer et al. (2002), like MPP^+, TaClo specifically inhibits the electron transfer from complex I to ubiquinone in the mitochondrial respiratory chain in both rat brain homogenate and rat liver submitochondrial particle preparations. TaClo was shown to reduce dopamine uptake and the number and size of tyrosine-hydroxylase-positive cells in C56/BL6 mouse primary cell cultures. Injection of TaClo directly into the rat substantia nigra pars compacta decreases both neuronal density and number of neurons in this region. This treatment also resulted in a progressive decline in concentrations of the dopamine metabolite 3,4-dihydroxyphenylacetic acid over 6 weeks after a single injection, consistent with some other models of the Parkinson's disease phenotype. While an intriguing series of studies, it is unknown whether TaClo can be formed following trichloroethylene exposure per se.

FINDINGS AND RECOMMENDATIONS

With respect to the EPA (2001b) draft risk assessment, several neurotoxicity studies contributed to the derivation of an inhalation RfC. In general, these studies report effects in humans and in experimental models (rat) at very similar concentrations. In addition, common effects are seen across these studies, and the nature of the effects described are comparable to or consistent with those reported in response to acute exposures to higher concentrations. Those studies utilized to derive the RfC include reports in humans of changes in trigeminal nerve function (measured using the massiter reflex latency) and motor incoordination at human equivalent LOAEL concentrations of 7-16 ppm (Ruijten et al. 1991; Rasmussen et al. 1993) and symptoms including nausea, drowsiness, and fatigue (Okawa and Bodner 1973; Vandervort and Polakoff 1973). Studies in rats showed changes in heart rate and wakefulness at a human pharmacokinetic adjusted LOAEL of 9 ppm (Arito et al. 1994). This appears to be a valid and standard approach taken to evaluate risk.

Furthermore, as is clear from the discussions above, new information on trichloroethylene published since the EPA (2001b) review is limited and thus may offer little in the way of amendment to the current RfC:

- The effects Ohta et al. (2001) described in mice after single intraperitoneal injections on long-term potentiation in hippocampal slices may be significant to any derivation of acute-exposure risk assessment, particularly considering the critical nature of the effect and its implications for complex cognitive function. However, the intraperitoneal route of administration makes extrapolation of these findings to humans difficult.
- Although the exposure concentrations (0.2-200 ppm) used in the subchronic study of rats by Poon et al. (2002) are low and thus would be of interest, small sample sizes used in components of this study may have precluded the ability to detect effects; histologic changes are hard to interpret given measurement at a single time point. Thus, the absence of effects reported in this study may reflect experimental inadequacies rather than representing actual no-observed-adverse-effect levels.
- The two studies by Kilburn (2002a,b) of chronic environmental exposures in humans have major limitations, including potential for bias, inconsistency of effects, absence of appropriate comparisons, and others as noted above. For these reasons, it is not clear that the studies can be used in the trichloroethylene risk assessment.
- A study by Reif et al. (2003) reporting that low (and high) concentrations of trichloroethylene affect memory, as well as a potentiation of such effects by alcohol (an interaction supported by the experimental literature) has limitations that include potential misclassification of exposures and bias, and thus must be interpreted with caution.
- Certainly of potential relevance to risk assessment are studies that suggest protracted effects of trichloroethylene after cessation of exposure, such as described by Oshiro et al. (2004) for dopaminergic systems and by Haglid et al. (1981) for alterations in protein levels in multiple brain regions.

One thing made clear by any assessment of the trichloroethylene literature as it relates to the nervous system is the paucity of data available to define the extent of its neurotoxicity and the parameters and conditions of exposure under which it occurs. For example, studies of chronic exposure are limited. The realities of evaluating the impact of human environmental exposures generally mean that measures of trichloroethylene exposures in such studies are estimated and not empirically determined, leaving open the possibility of misclassification, a problem that can increase or decrease the probability of detecting effects. In some other human studies, the effects are confounded by involvement of the subjects in litigation. When experimental studies were carried out, they were generally not lifetime studies and the extent to which they evaluated behavioral and neurological function was limited, because most of the emphasis was on carcinogenicity.

Recommendation: Long-term studies, human and experimental, are critically needed to evaluate the effect of trichloroethylene on the central nervous system. For human studies, measurement or better estimates of exposure are necessary.

Another gap in the literature is the extent to which development represents a period of enhanced susceptibility to the neurotoxic effects of trichloroethylene. The only comparative data available at the current time appears to be from the study of Moser et al. (1999) examining one metabolite of trichloroethylene, dichloroacetic acid, where the evidence in support of enhanced susceptibility of younger rats is limited. Statements of greater sensitivity of children than of

adults exposed to trichloroethylene are also described by White et al. (1997), but again the supporting data are not presented.

The one study published to date clearly demonstrates an enhanced sensitivity of aging rats relative to young rats to the effects of trichloroethylene, measured in that study as changes in heart rate (Arito et al. 1994). These changes were shown to be due to changes in pharmacokinetics with age, with older rats exhibiting higher brain concentrations of trichloroethylene as well as longer exposure (delayed clearance) to those doses. One would predict that this enhanced toxicity should generalize to other behavioral and neurological consequences of trichloroethylene as well, because functionally it represents a higher dose to the brain, particularly if peak blood trichloroethylene concentrations are critical to adverse effects (Boyes et al. 2003). This also means that aging and exposure duration may be related in chronic exposure scenarios in humans.

Recommendation: More research is needed to assess different life stages at which humans might be more susceptible to the neurotoxic effects of trichloroethylene.

Another area of interest is the possibility of permanency versus reversibility of effects and the conditions under which this could occur. To date, the evidence is conflicting and undoubtedly would reflect the parameters of exposure, but some studies document protracted effects of trichloroethylene on the nervous system (e.g., Haglid et al. 1981; Oshiro et al. 2004; Crofton et al. 1993). It is clear from studies reported many years ago that acute exposures to high concentrations of trichloroethylene, in occupational or experimental contexts, can produce permanent changes in the nervous system. What is not yet known is the exposure conditions, particularly repeated exposures, under which effects would no longer be reversible. A related issue is whether effects of exposure can be progressive even after that exposure has terminated.

Recommendation: Additional research examining the extent to which observed effects are permanent versus reversible would be of releavance to risk evaluation.

Despite the associations of occupational exposures with memory loss and other cognitive deficits, the nature of such effects and the exposure conditions with which they can be associated have not been elaborated. A report by White et al. (1997), despite its deficiencies, clearly shows common effects on complex cognitive functions across three different populations exposed to trichloroethylene environmentally. Experimental studies have been less clear about such effects, but the extent to which this has been addressed is limited and, in some published reports, is not interpretable with respect to outcome.

A related function that clearly seems to be affected by trichloroethylene is motor function, as has been demonstrated in experimental studies as well as in occupational cohorts. As with other behavioral functions, the trichloroethylene exposure conditions under which such effects occur are not yet known. It may be important to define such conditions, particularly if, as suggested by other reports, trichloroethylene might contribute to neurodegenerative disorders such as Parkinson's disease. The earliest signs of motor dysfunction could serve as biomarkers of such a contribution.

Many neurological and behavioral disorders represent complex multifactorial etiologies. Given the broad spectrum of its effects across behavioral domains as well as neurotransmitter systems (and other as yet unknown mechanisms), it is possible that trichloroethylene may

contribute as a risk factor to other neurodegenerative and behavioral diseases or dysfunctions, acting in conjunction with other risk modifiers that may include genetic background (P-450 polymorphisms) and lifestyle factors (e.g., alcohol consumption), aging, and other factors that are undetermined.

Recommendation: Studies of additional functional end points, including cognitive deficits and motor and sensory function in response to chronic exposures to trichloroethylene would be of value to risk assessment.

Current evidence suggests the possibility of multiple mechanisms by which trichloroethylene may act, with the recognition that these mechanisms may also depend on the parameters of exposure. Extant literature already documents changes in long-term potentiation as well as alterations in functions of several neurotransmitter systems, a basis from which complex cognitive functions as well as other behavioral domains could be impaired.

Recommendation: Additional research is required to elucidate the underlying modes of action of trichloroethylene-induced neurotoxicity.

7

Respiratory Tract Toxicity and Cancer

This chapter reviews information on the effects of trichloroethylene on the respiratory system, particularly information generated since the U.S. Environmental Protection Agency released its draft health risk assessment (EPA 2001b). In keeping with the committee's charge, the chapter focuses on hazard characterization and the mode of action for trichloroethylene toxicity, assessing the available information from in vitro, animal, and human studies. Cancers of the respiratory tract are also considered.

RESPIRATORY TRACT TOXICITY

In Vitro Studies

Trichloroethylene, tested in swine trachea in vitro for its effects on smooth muscle contraction and epithelial release of prostanoids, did not alter the basal tone of tracheal smooth muscle but did potentiate the muscle contractile responses to acetylcholine and histamine in a concentration-dependent manner. Trichloroethylene increased epithelial prostaglandin E_2 release and decreased acetylcholinesterase activity. Such responses are consistent with the reported effects of trichloroethylene exposure on increasing airway hyperresponsiveness and asthma (Chen et al. 2005).

In Vivo Studies

The time course of trichloroethylene-induced pulmonary injury was followed in CD-1 male mice exposed to [^{14}C]trichloroethylene in a single oral dose of 2,000 mg/kg (Forkert and Birch 1989). Clara cells of the bronchiolar epithelium showed necrotic changes within 1 hour of dosing and most Clara cells were severely vacuolated by 24 hours. Twenty percent of the lung burden at 4 hours was covalently bound. The study indicates that the highly metabolic Clara cells are targets of trichloroethylene toxicity in the respiratory tract.

Human Studies

There are few reports of non-cancer pulmonary toxicity in trichloroethylene-exposed humans. A study of respiratory findings in gun factory workers exposed to multiple solvents indicated significant effects of smoking and exposure to solvents, with smoking having the most important effect on asthma-related symptoms. Trichloroethylene was only one of many solvents to which the workers were exposed (Cakmak et al. 2004).

Toxicokinetics and Mode of Action

Pulmonary toxicity induced by trichloroethylene is associated with cytochrome P-450-dependent bioactivation to reactive metabolites (see Figure 7-1). The predominant pathway of

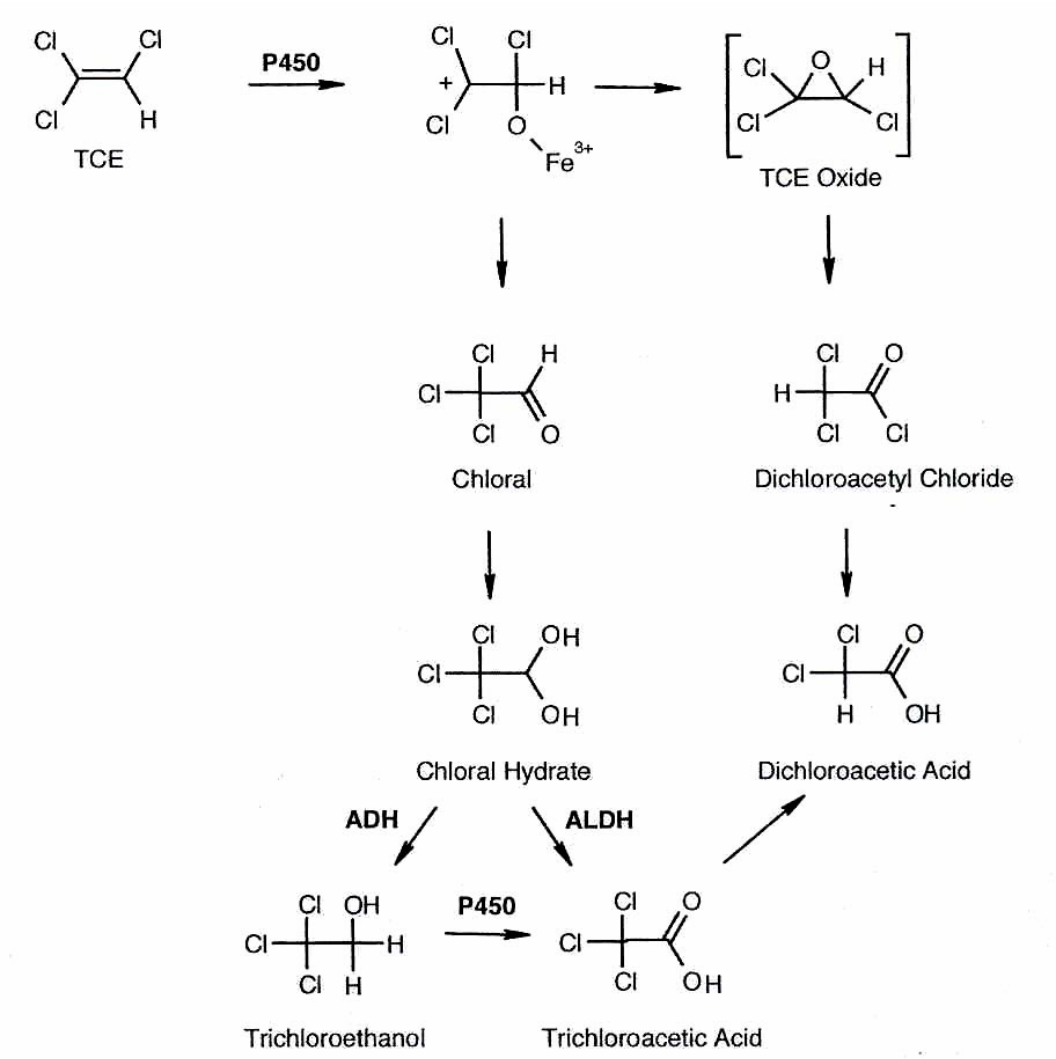

FIGURE 7-1 Proposed scheme of trichloroethylene metabolism. Abbreviations: TCE, trichloroethylene; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase. Source: Forkert et al. 2005. Reprinted with permission; copyright 2005, American Society for Pharmacology and Experimental Therapeutics.

trichloroethylene metabolism is oxidation via the cytochrome P-450 system, mainly by CYP2E1, although other P-450 enzymes including CYP1A1/2, CYP2B1/2, CYP2C11/6, and CYP2F have been implicated (Guengerich et al. 1991; Nakajima et al. 1992a; Forkert et al. 2005). Oxidative metabolism of trichloroethylene yields the primary metabolites chloral, trichloroethylene oxide, and dichloroacetyl chloride. Chloral, a predominant metabolite of trichloroethylene, is rapidly converted to chloral hydrate, which then undergoes oxidation and reduction by aldehyde-dehydrogenase and alcohol-dehydrogenase enzymes to form trichloroacetic acid and trichloroethanol (Green and Prout 1985; Dekant et al. 1986b). Clara cells isolated from the mouse lung are known to efficiently metabolize trichloroethylene to chloral and trichloroacetic acid. Recent studies of recombinant (r) cytochrome P-450s and rodent and human lung microsomes revealed that rat and human rCYP2E1, rCYP2F, and rCYP2B1 were all capable of mediating trichloroethylene metabolism to chloral hydrate (Forkert et al. 2005). Rat rCYP2E1 exhibited greater affinity than rat rCYP2F4 and rCYP2B1 and human rCYP2E1. More recently, the same investigators (Forkert et al. 2006) suggested that CYP2F2 might play a greater role than CYP2E1 in the metabolism of trichloroethylene in the mouse lung. Treatment of CYP2E1-null and wild-type mice with trichloroethylene led to bronchiolar damage that correlated with the formation of dichloroacetyl adducts in the Clara cells. These findings provide evidence for bioactivation of trichloroethylene within the Clara cells, predominantly involving CYP2F2, that correlates with bronchiolar cytotoxicity.

The rates of chloral hydrate production in human lung microsomes were low and were detected in only three of eight subjects (Forkert at al. 2005). Furthermore, the rates of chloral hydrate production were substantially higher in murine than in human lung. Alcohol dehydrogenase, the enzyme responsible for metabolizing chloral to trichloroethanol, is known to be at low concentrations in lung tissue (Sorokin 1970), with chloral being the major metabolite in these cells. Trichloroethanol glucuronide, a major metabolite in the liver, is not formed in the Clara cells due to lack of glucuronyltransferase (Odum et al. 1992). Recent studies of mice treated intraperitoneally with high doses (500-1,000 mg/kg) of trichloroethylene found dichloroacetyl protein adducts in Clara cells (Forkert et al. 2006). The proximate toxicant for the Clara cell, whether chloral, dichloroacetyl chloride, or another metabolite, is still under study.

Exposure to trichloroethylene occurs mainly through inhalation and oral routes and rapid absorption occurs by both routes. Absorption of inhaled trichloroethylene is both rapid and extensive. Regardless of the route of exposure, unmetabolized trichloroethylene is eliminated by exhalation. Consequently, pulmonary airways are exposed to trichloroethylene regardless of the route of exposure. However, the amount of pulmonary exposure to trichloroethylene after an oral exposure is dose dependent and will be high only after an oral dose exceeds the capacity of the liver to metabolize the trichloroethylene. After inhalation exposure, trichloroethylene is rapidly absorbed through the alveolar endothelium due to a high blood-gas partition coefficient. However, the blood-gas partition coefficient in humans is 1.5- to 2.5-fold lower than that in mice and rats, respectively, which suggests that delivery of trichloroethylene to the circulatory system for translocation to target organs may be significantly less efficient in humans. This factor should be taken into account when using animal data in risk assessment analysis for trichloroethylene (Sato et al. 1977; Prout et al. 1985; Clewell et al. 1995).

The Clara cells develop vacuoles (Forkert and Birch 1989; Odum et al. 1992) after exposure to trichloroethylene and proliferate with continued exposure (Green et al. 1997b). Considering the site of induced tumors in mice and the observed toxicity sites, it appears that the Clara cell is the most sensitive site in the respiratory tract with respect to the toxicity of inhaled

trichloroethylene. CYP2E1 and CYP2F2 are highly concentrated in mouse Clara cells (Buckpitt et al. 1995; Forkert et al. 1995) and the presence of dichloracetyl protein adducts in these same cells (Forkert et al. 2006) suggests that the bioactivation of trichloroethylene takes place in these cells. The low CYP2E1 concentration in human Clara cells suggests that humans are not as sensitive as mice for the development of lung tumors as a result of trichloroethylene exposures at ambient levels. This hypothesis agrees with the results of most epidemiology studies (discussed later in this chapter), which do not indicate a strong association between trichloroethylene exposure and increased incidence of lung tumors.

Whereas trichloroethylene is both acutely toxic and carcinogenic to the mouse lung after exposure by inhalation, it is not carcinogenic in the rat lung and is markedly less toxic after acute exposure (Stewart et al. 1979; Fukuda et al. 1983; Maltoni et al. 1986, 1988; Davidson and Beliles 1991). The lack of toxicity or carcinogenicity in the lungs of mice after oral dosing is presumably due to extensive hepatic metabolism reducing the amount of trichloroethylene that reaches the lungs. Adverse effects on human lungs have not been reported. The primary effects of trichloroethylene on mouse lungs in all studies have been morphologic and biochemical changes in the nonciliated Clara cells (Forkert 2001). The only other toxicologic responses noted in the lung after exposure to trichloroethylene were fibrosis in the mouse (Forkert and Forkert 1994) and a decrease in surfactant phospholipid after exposure of rats and mice to high doses (3,000 mg/kg) of trichloroethylene (Scott et al. 1988). Loss of cytochrome P-450 activity and morphologic recovery of the Clara cells with repeated daily exposure of mice to trichloroethylene suggest that loss of metabolic capacity in these cells is an adaptive mechanism (Lewis et al. 1984; Forkert et al. 1985, 2005; Odum et al. 1992).

RESPIRATORY TRACT CANCER

Animal Studies

Animal carcinogenicity studies are summarized in Table 7-1. Trichloroethylene inhalation exposure caused an increased incidence of pulmonary tumors in mice (Fukuda et al. 1983; Maltoni et al. 1986, 1988) but not in rats and hamsters (Henschler et al. 1980; Fukuda et al. 1983; Maltoni et al. 1986, 1988). Oral administration of trichloroethylene did not result in lung tumors (NCI 1976; Van Duuren et al. 1979; Henschler et al. 1984; Maltoni et al. 1986; NTP 1988, 1990) in any species studied. Details of those studies follow.

Inhalation Exposure

The inhalation studies by Fukuda et al. (1983) included exposing female ICR mice and female Sprague-Dawley rats to trichloroethylene at 0, 50, 150, or 450 ppm for 7 hours/day, 5 days/week for 104 weeks followed by an observation period of 3 weeks. They observed a threefold increase in lung tumors per mouse in those exposed to the two higher concentrations but saw no increase in lung tumors in the rats.

In the studies by Maltoni et al. (1986, 1988), male and female Swiss mice and Sprague-Dawley rats were exposed to trichloroethylene at 0, 100, and 600 ppm for 7 hours/day, 5 days/week for 8 weeks and for 104 weeks (rats only). $B6C3F_1$ and Swiss mice were also

TABLE 7-1 Animal Carcinogenicity Studies of Trichloroethylene

Reference	Animals (Sex)	Exposure Route	Stabilizers	Doses/Exp Conc.	Exposed	Results
Fukuda et al. 1983	ICR mice (F) S-D rats (F)	Inhalation, 7 h/day, 5 days/week, 104 wk, hold 3 wk	Epichlorohydrin	0, 50, 150, 450 ppm	50/group	Threefold increase in lung tumors in mice at two higher concentrations; no increase in any tumors in rats.
Maltoni et al. 1986, 1988	S-D rats (M, F)	Inhalation, 7 h/day, 5 days/week, 104 wk; hold until death	No stabilizer	0, 100, 300, 600 ppm	130-145/ group	Increase in tumors of testis and kidney (high dose only) in males.
	Swiss mice (M, F) B6C3F1 mice	Inhalation, 7 h/day, 5 days/week, 78 wk; hold until death	No stabilizer	0, 100, 300, 600 ppm	90/group	Excess lung tumors in both strains; liver tumors in male Swiss mice.
Henschler et al. 1980	Wistar rats (M, F) Syrian hamsters (M, F) NMRI mice	Inhalation, 6 h/day, 5 days/week, 18 months	Triethanolamine	0, 100, 500 ppm	30/group	No increase in any tumors, except increase in lymphomas in female mice.
Van Duuren et al. 1979	Swiss mice (M, F)	Gavage, 1/wk, 89 wk	Unknown	0, 0.5 mg	30/group	No excess tumors in lung, liver, or stomach.
NCI 1976	Osborne-Mendel rats (M, F) $B6C3F_1$ mice (M, F)	Gavage, 5/wk, 78 wk, hold 110 wk (rats) or 90 wk (mice)	Epoxybutane, epichlorohydrin	0, 500, 1,000 mg/kg (rats) 0, 1,000, 3,000 mg/kg (mice)	50/group except 20/control	No excess pulmonary tumors; increase in liver tumors.
NTP 1988	4 strains of rats	Gavage, 1/day, 5 days/week, 103 wk	No stabilizer	0, 500, 1,000 mg/kg	50/group	No excess pulmonary tumors; some renal and testicular tumors in two strains.
NTP 1990	F344 rats (M, F) $B6C3F_1$ mice (M, F)	Gavage, 1/day, 5/wk, 103 wk	No stabilizer	0, 500, 1,000 mg/kg (rats) 0, 1,000 mg/kg (mice)	50/group	No pulmonary tumors; increase in liver tumors.
Maltoni et al. 1986	S-D rats (M, F)	Gavage, 1/day, 4-5 days/week, 56 wk; hold until death	No stabilizer	0, 50, or 250 mg/kg	30/group	No excess tumors.

Abbreviations: F, female; M, male; S-D, Sprague-Dawley.

exposed for 78 weeks to trichloroethylene at 0, 100, 300, or 600 ppm. Animals were held for observation until spontaneous death. Excess tumors were not observed in either species after the 8-week exposures. Excess pulmonary tumors were observed in both strains of mice after 78 weeks of exposure, but no excess pulmonary tumors were observed in rats after the 104-week exposures.

Henschler et al. (1980) exposed NMRI mice, WIST rats, and Syrian hamsters of both sexes to trichloroethylene at 0, 100, or 500 ppm for 6 hours/day, 5 days/week for 18 months. They observed no pulmonary tumors in any of the species. The trichloroethylene used was technical grade and contained traces of epichlorohydrin and 1,2-epoxybutane—two known carcinogenic compounds used as stabilizers.

Gavage Exposure

Van Duuren et al. (1979) tested trichloroethylene among 14 other halogenated compounds for carcinogenicity in Swiss mice. The mice (both sexes) were administered trichloroethylene at 0.5 mg per intragastric intubation once a week for 622 days (89 weeks). No excess tumors were observed.

The National Cancer Institute (NCI 1976) and the National Toxicology Program (NTP 1988, 1990) reported three carcinogenicity studies of trichloroethylene administered by gavage to rats and mice. The first study (NCI 1976) used both sexes of Osborne-Mendel rats and $B6C3F_1$ mice. Animals were dosed with trichloroethylene at approximately 500 or 1,000 mg/kg (rats) or approximately 1,000 or 2,000 mg/kg (mice), 5 times/week for 78 weeks. Rats were observed for 110 weeks and mice were observed for 90 weeks. No increase in pulmonary tumors was observed in either species, but the study was not considered valid because of early mortality among the rats. The trichloroethylene was technical grade and was stabilized with epichlorohydrin and 1,2-epoxybutane. In a later study (NTP 1988), trichloroethylene stabilized by diisopropylamine was administered orally to four strains of rats at 0, 500, or 1,000 mg/kg per day, 5 days/week, for 103 weeks. No pulmonary tumors were observed, but the study was considered inadequate because of chemical toxicity and early mortality in the rats. The third study (NTP 1990) was conducted in male and female F344 rats and $B6C3F_1$ mice with epichlorohydrin-free trichloroethylene. Doses of 500 or 1,000 mg/kg in rats and 1,000 mg/kg in mice were given by gavage 5 days/week for 103 weeks. No pulmonary tumors were observed. The study was considered adequate for demonstrating no carcinogenicity in female rats, but the male rats did not survive long enough to test adequately for carcinogenicity.

Epidemiology Studies

Because it was not possible to provide a comprehensive evaluation of the epidemiologic evidence on trichloroethylene and different cancers, the committee borrowed a previously compiled summary of the epidemiologic evidence on lung cancer from the Institute of Medicine (IOM 2003) to give some perspective on the evidence for lung cancer (see Table 7-2). The list was updated with one study published since the IOM report. Epidemiology studies do not indicate an increase in pulmonary tumors in association with trichloroethylene exposure, except for the new study in Denmark (Raaschou-Nielsen et al. 2003), which was done on a cohort of

TABLE 7-2 Epidemiologic Data on Lung Cancer and Exposure to Trichloroethylene

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% confidence interval)
Cohort Studies—Incidence			
Raaschou-Nielsen et al. 2003	Workers in Denmark		
	Males		
	<1 yr	181	1.6 (1.4-1.9)
	1-4.9 yr	193	1.3 (1.1-1.5)
	≥5 yr	185	1.4 (1.2-1.6)
	Females		
	<1 yr	28	2.5 (1.6-3.6)
	1-4.9 yr	25	1.6 (1.1-2.4)
	≥5 yr	20	1.6 (1.0-2.5)
Hansen et al. 2001	Biologically monitored workers in Denmark		
	Males, ever exposed	16	0.8 (0.5-1.3)
	Females, ever exposed	1	0.7 (0.01-3.8)
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Males		
	No exposure	22	1.0 (0.5-1.9)
	<5 unit-yr	24	1.0 (0.6-2.0)
	5-25 unit-yr	11	0.8 (0.4-1.6)
	>25 unit-yr	15	0.8 (0.4-1.7)
	Females		
	No exposure	0	—
	<5 unit-yr	1	0.6 (0.1-5.3)
	5-25 unit-yr	0	—
	>25 unit-yr	0	—
Anttila et al. 1995	Biologically monitored workers in Finland		
	Entire period since first measurement	25	0.92 (0.59-1.35)
	0-9 yr	11	1.19 (0.59-2.13)
	10-19 yr	9	0.67 (0.30-1.26)
	≥20 yr	5	1.11 (0.36-2.58)
	Mean personal U-TCA concentration		
	<100 μmol/L	16	1.02 (0.58-1.66)
	100+ μmol/L	7	0.83 (0.33-1.71)
Cohort Studies—Mortality			
Boice et al. 1999	Aircraft-manufacturing workers in California		
	All exposed factory workers	78	0.76 (0.60-0.95)
	Duration of potential exposure (routine or intermittent)		
	<1 yr	66	0.85 (0.65-1.13)
	1-4 yr	63	0.98 (0.74-1.30)
	≥5 yr	44	0.64 (0.46-0.89)
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Males		
	No exposure	51	1.0 (0.7-1.6)
	<5 unit-yr	43	1.0 (0.6-1.6)
	5-25 unit-yr	23	0.9 (0.5-1.6)
	>25 unit-yr	38	1.1 (0.7-1.8)

TABLE 7-2 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% confidence interval)
	Females		
	No exposure	2	0.4 (0.1-1.6)
	<5 unit-yr	2	0.6 (0.1-2.4)
	5-25 unit-yr	11	0.6 (0.1-4.7)
	>25 unit-yr	2	0.4 (0.1-1.8)
Morgan et al. 1998	Aerospace workers in Arizona		
	Entire TCE-exposed cohort	97	1.10 (0.89-1.34)
	Cumulative		
	Low	45	1.49 (1.09-1.99)
	High	52	0.90 (0.67-1.20)
	Peak: medium and high versus low and no exposure	64	1.07 (0.82-1.40)
Greenland et al. 1994	White male transformer-assembly workers, ever exposed	NA	1.01 (0.69-1.47)
Wilcosky et al. 1984	White male rubber-industry workers in Ohio, cumulative exposure of more than 1 yr	11	0.64

Abbreviations: NA, not available; TCE, trichloroethylene; U-TCA, urinary trichloroacetic acid.
Source: Adapted from IOM 2003.

more than 40,000 blue-collar workers in 347 Danish companies where trichloroethylene was used. The standardized incidence ratio did not increase with duration of exposure, and was highest among workers with less than 1 year of exposure, particularly among females (SIR = 2.5). The authors suggested that smoking might be a confounding factor for cancers known to be related to tobacco use because of the lower socioeconomic status of the cohort and the higher prevalence of smoking among the least educated groups in Denmark. The authors also estimated that only 41% of the cohort were likely to have been exposed to trichloroethylene on the job, because the only marker of exposure used was employment in a blue-collar job at a trichloroethylene-using company. The large excess of lung cancer among females with the shortest exposures argues strongly against a causal interpretation for these findings.

Mode of Action

When trichloroethylene is inhaled, a large portion is absorbed (a greater percentage of what is inhaled is absorbed at lower concentrations) and is rapidly distributed throughout the body in the bloodstream. The liver is the major site of metabolism, but the fact that inhaled trichloroethylene causes lung tumors in mice, whereas orally administered trichloroethylene does not, suggests that pulmonary metabolism may play a role in lung tumor formation in mice. Trichloroethylene is not mutagenic but some of its metabolites are. Because trichloroethylene is lipid soluble, one would expect the compound to be delayed long enough in crossing the lung tissue into the blood to be partially metabolized by metabolically active epithelial cells (Clara cells and Type II cells) (Gerde et al. 1993). The metabolic activity of such cells toward several lipophilic xenobiotics is known to be higher in mouse cells than in rat or hamster cells, which

may be part of the reason for the greater susceptibility of mouse tissue than of tissue in rats and hamsters.

Some investigators have reported that the capacity of the mouse lung to metabolize trichloroethylene to the mutagenic metabolite chloral is an order of magnitude higher than in the rat lung, whereas human lung samples have no detectable activity (Green et al. 1997b). The CYP450 (2E1) enzyme responsible for this oxidation is present in high amounts in the Clara cells of mice and in lesser amounts in Clara cells of rats. The enzyme was not detected in human Clara cells, although mRNA for the enzyme has been detected and variable amounts of CYP2E1-like metabolism has been observed in human lung microsomes (Forkert et al. 2001)..

The Clara cell is the site of toxicity induced by inhalation of trichloroethylene in mice. A number of acute toxicity studies have shown that trichloroethylene selectively targets the mouse lung Clara cell, suggesting the role of Clara cells in the development of mouse lung tumors (Buckpitt et al. 1995; Forkert and Forkert 1994). Although there is no direct evidence that mouse lung tumors are derived from Clara cells, the lack of other types of epithelial cells such as Type II cells responding by cell division suggests that Clara cells play a key role in the development of lung tumors in mice exposed to trichloroethylene. Similarly, differences in the metabolic capacity of Clara cells in mice and rats are consistent with species differences in toxicity and carcinogenicity (Green 2000). Clara cells are thought to be the cells of origin of some chemically induced mouse lung tumors (Sorokin 1970; Kauffman et al. 1979; Sato and Kauffman 1980; Kauffman. 1981; Thaete and Malkinson 1991; Palmer. 1985; Rasmussen et al. 1986; Rehm et al. 1991). However, evidence from antigenic staining for detailed morphologic characterization of the tumors is lacking.

ISSUES

Possible Modes of Action for Cancer and Non-Cancer Effects

Cytotoxicity and increased cell division form the basis of a plausible mode of action for trichloroethylene-induced lung tumors in mice. Both are known risk factors for mouse lung carcinogenesis because of significant background tumor incidences (Green 2000). In addition, chloral appears to have genotoxic potential (Salmon et al. 1995), although in the whole lung two studies failed to find evidence of DNA binding in mice exposed to trichloroethylene (Forkert and Birch 1989; Leuschner and Leuschner 1991). In conclusion, a number of known risk factors for the development of tumors, such as cytotoxicity, increased cell proliferation, and possibly aneuploidy, correlate well with the observed species-specific pulmonary carcinogenicity of trichloroethylene.

Relevance of Animal Studies to Humans

The acute responses believed to be causally related to the development of lung tumors in mice exposed to trichloroethylene have been attributed to the high metabolic capacity of the mouse lung Clara cells. Comparisons of the metabolic capacity of mouse, rat, and human lung tissue found that mouse lung microsomes metabolized trichloroethylene to chloral at a rate three-fold higher than the rat lung microsomes. A metabolic rate could not be detected in the human

lung (Green et al. 1997b). With an antibody to CYP2E1, the enzyme responsible for metabolism of trichloroethylene to chloral (Green et al. 1997b; Cruzan et al. 1997), the highest concentrations of the enzyme were found in the mouse lung. Significantly lower amounts were found in the rat lung. This enzyme could not be detected in the human lung in any cell types or in human lung sections by Western blotting (Green et al. 1997b). Other studies (Guengerich et al. 1991; Wheeler et al. 1992; Willey et al. 1996) reported the presence of CYP2E1 in human lung detectable only by reverse transcriptase-polymerase chain reaction. The total cytochrome P-450 content of the human lung is reported to be only 3.7% (27-fold lower than) that in the rat lung (Raunio et al. 1998). This is consistent with the lack of a measurable metabolic rate for trichloroethylene (Green et al. 1997b). Studies with recombinant cytochrome P-450s have revealed that, although rat and human rCYP2E1, rCYP2F, and rCYP2B1 were all capable of mediating trichloroethylene metabolism to chloral hydrate, the rat rCYP2E1 exhibited greater affinity than rat rCYP2F4 and rCYP2B1 and human rCYP2E1 (Forkert et al. 2005). These studies provide evidence supporting the role of CYP2E1, CYP2F, and CYP2B1 in the metabolism of trichloroethylene in the mouse lung. Studies with mouse and human lung microsomes indicated that the rates of chloral hydrate formation in human lung microsomes were low and were detected in only three of eight subjects (Forkert at al. 2005). Overall, the data suggest that the capacity of the human lung to metabolize trichloroethylene is 600-fold lower than the mouse lung (Green 2000).

The number of Clara cells and their morphology differ in human and rodent lungs, and this should also be considered in evaluating human risk from exposure to trichloroethylene. Clara cells differ significantly between rodents and among rodents and humans in number and structure (Reznik-Schuler 1976; Buckpitt et al. 1995). In mice, Clara cells are numerous and are spread throughout the airways, whereas in rats they are significantly fewer, particularly in the terminal bronchiolar region. In human lung, Clara cells are rare, as they are found in small numbers in the distal bronchioles. Mouse lung Clara cells are packed with endoplasmic reticulum, but human Clara cells are devoid of these membranes (Reznik-Schuler 1976; Buckpitt et al. 1995). The membranes of the endoplasmic reticulum in the Clara cell are the site of origin of the trichloroethylene-induced lesion in the mouse, consistent with the location of high concentrations of cytochrome P-450 enzymes that metabolize trichloroethylene in those membranes.

In summary, the metabolic data suggest that humans will be much less sensitive than mice to trichloroethylene-induced Clara cell toxicity and lung tumor development. One would not expect humans to develop lung tumors after exposure to ambient levels of trichloroethylene, which is in agreement with the results of epidemiology studies that show no association between trichloroethylene exposures and an increased in incidence of lung tumors.

FINDINGS AND RECOMMENDATIONS

Trichloroethylene has been shown to induce lung tumors in rodents. It is well documented that the mode of action for this effect is localization of the metabolite chloral in Clara cells of the lungs and that pulmonary metabolism of trichloroethylene to chloral is species dependent. The weight of evidence indicates that rodents and humans differ significantly in their capacity to metabolize trichloroethylene in their lungs, with humans having less capacity to metabolize the compound. This is supported by the results of most epidemiologic studies of

occupational exposure to trichloroethylene, which do not show a strong association between trichloroethylene exposure and an increased incidence of lung tumors. Thus, pulmonary cancer is does not appear to be a critical end point in assessing human health risks of trichloroethylene.

8

Immunotoxicity

This chapter reviews information about the effects of trichloroethylene on the immune system, particularly information generated since the U.S. Environmental Protection Agency released its draft health risk assessment (EPA 2001b). Consideration is given to how the new information factors into previous assessments of the immunosuppressive and autoimmune effects of trichloroethylene, species differences, dose-response relationships, and mode of action information.

BACKGROUND

Immunotoxicity can be divided into two areas depending on whether the immune system is activated (such as in allergies or in chemical-induced autoimmune diseases) or suppressed by xenobiotics (foreign or nonendogenous chemicals, including drugs and environmental chemicals). Mammalian immune systems have innate and adaptive components that play important roles in resistance to infections and cancer. The immune systems of mammals are formed by primary lymphoid organs, including yolk sac, fetal liver, bone marrow, and thymus. Secondary lymphoid organs (e.g., lymph nodes, spleen, mucosa-associated lymphoid tissues) store differentiated cells that await activation by environmental antigens or undergo endogenous selection processes to discriminate self from non-self. T and B cells are activated in clonally restricted (antigen-specific) ways, and they demonstrate a memory response. One feature of innate immunity is that the responding cells (macrophages, natural killer cells, granulocytes) do not demonstrate clonal specificity. However, families of receptors have been identified (such as toll-like receptors) that allow innate cells to respond to certain families of environmental molecules or toxins (e.g., endotoxin). Xenobiotics may interfere with normal immune system homeostasis by affecting the formation of immune cells; modifying cell-to-cell interactions; modifying cell activation, proliferation, or differentiation; altering cell selection; and enhancing or suppressing the release of immune products such as cytokines, chemokines, antibodies, and complement factors.

The immunotoxicity of chemicals is evaluated in animal models, in in vitro studies, and occasionally in humans after occupational or environmental exposures. Environmental

epidemiology studies are often conducted to determine whether xenobiotic exposures are associated with disease. Because of the complexity of the innate and adaptive immune systems, no single assay can be used to study the potential toxicity of xenobiotics. Instead, a tiered approach has been developed and validated by several laboratories for studies in animals (Luster et al. 1988, 1992). Although there is no single immune assay or parameter that can be used to determine whether a xenobiotic exerts a toxic effect on the immune system, certain combinations of markers and functional assays can predict immunotoxicity (Luster et al. 1992). Additionally, the aforementioned assays are useful only for evaluating immunosuppressive chemicals. Few established assays exist for assessing hypersensitivity reactions of xenobiotics, and experimental models of autoimmunity are limited in their application and extrapolation to human autoimmune diseases.

ANIMAL STUDIES

Immunosuppression

The potential immunosuppressive and immunomodulating properties of trichloroethylene in acute, subchronic, and chronic exposures in animals have not been fully evaluated. Sanders et al. (1982) found that trichloroethylene at concentrations of 2.5-5 mg/mL (in drinking water for 4 or 6 months) resulted in suppression of humoral and cell-mediated immunity in female CD1 mice. Bone marrow stem cell activity was depressed at drinking water concentrations of 0.1-1 mg/mL. Male mice were less affected. Wright et al. (1991) found a depression in natural killer cell activity in the liver, decreased lipopolysaccharide lymphocyte mitogenesis, and decreased spleen weights after intraperitoneal exposures of Sprague-Dawley rats to trichloroethylene at 5 mmol/kg/day for 3 days. Natural killer cell activity in the liver was also depressed at 0.5 mmol/kg/day for 3 days. $B6C3F_1$ mice receiving the high-dose regimen also demonstrated spleen cell toxicity, and they were more sensitive than rats to the natural killer cell suppression in the liver, with effects observed at 0.05 mmol/kg/day for 3 days. Aranyi et al. (1986) found that acute exposures to various solvents decreased the host resistance responses to *Klebsiella pneumoniae*. In these studies, trichloroethylene was not evaluated, but a related solvent, perchlorethylene, had a small effect. Kauffmann et al. (1982) found that mice exposed to chloral hydrate at 1/10th (144 mg/kg over 14 days) and 1/100th of a median lethal dose (14.4 mg/kg over 14 days) had no changes in immune parameters. However a 90-day exposure to trichloroethylene at 0.07 and 0.7 mg/mL in drinking water produced a significant decrease in humoral immunity in female, but not male, mice. Park et al. (1993) found that trichloroethylene at 50-200 parts per million (ppm) increased infection in bacteria-challenged (*Streptococcus zooepidimicus*) mice.

Kaneko et al. (2000) determined that inhalation of high concentrations of trichloroethylene (500-2,000 ppm) for 8 weeks depressed the serum IgG in *mrl/lpr* mice and increased the formation of lymphoblastoid cells. Changes in T-cell subsets (helper to suppressor ratio) were detected at 2,000 ppm after 8 weeks of exposure. The significance of these findings is difficult to assess because the investigators used an autoimmune-prone mouse strain (*mrl*/*lpr*), which is not commonly used in studies of immunosuppression.

In summary, various studies indicate that exposures to moderate or high concentrations of trichloroethylene over long periods have the potential to produce immunosuppression in animal

models. There are important differences in the amounts and types of immunosuppression depending on species and gender.

Autoimmunity

Epidemiology and case studies revealed that solvents, including trichloroethylene, might be associated with certain human autoimmune diseases. These reports triggered investigators to evaluate the effect of trichloroethylene in animal models susceptible to the induction of autoimmune disease, most notably the MRL mouse. Autoimmune disease has not been reported in normal mice treated with trichloroethylene, although a small increase in autoantibodies has been noted in some studies (see below).

Findings in Rodents

Several laboratories have reported that trichloroethylene causes or exacerbates underlying autoimmune diseases in genetically susceptible MRL mice. Effects have been observed at doses as small as 0.1 mg/kg/day in drinking water for 4 weeks (Griffin et al. 2000a), which the authors calculated may be below the current threshold limit value of 50 ppm set by the American Conference of Industrial Hygienists. Recently, extensive mechanistic work has been performed and several biologically plausible hypotheses have been advanced (Khan et al. 1995, 2001; Gilbert et al. 1999, 2004; Griffin et al. 2000a,b,c; Blossom et al. 2004). Several studies focused on the need for metabolism of trichloroethylene to chloral or dichloroacetic acid to produce autoimmune-induced hepatitis in genetically susceptible mice (Griffin et al. 2000a,b) (see Chapter 4), a syndrome that bears potential mechanistic similarities to halothane-induced hepatotoxicity in rodents and humans. Evidence has been presented that trichloroethylene or its metabolites may activate T cells (Gilbert et al. 1999, 2004; Griffin et al. 2000a) and/or alter T cell regulation and survival (Blossom et al. 2004) associated with polyclonal disease, as detected by circulating anti-DNA and other antibodies in genetically susceptible mice. Theoretically, trichloroethylene metabolites may be increased with enhancers of the *CYP2E1* gene, and autoimmunity in MRL mice induced by trichloroethylene has been shown to be inhibited with *CYP2E1* inhibitors (Griffin et al. 2000b). Despite these hypotheses, the mechanism(s) by which trichloroethylene exacerbates autoimmune disease in MRL mice has not been elucidated and the relevance to human exposures and disease has not been established.

Gilkeson et al. (2004) administered trichloroethylene at 1,000-10,000 parts per billion (ppb) in drinking water to NZB/NZW mice for 26 weeks. They found an increase in anti-DNA antibodies with trichloroethylene at 1,000 ppb and an increase in kidney disease at 10,000 ppb. In the same study, $B6C3F_1$ mice developed a small increase in autoantibody production, but no kidney disease was detected.

White et al. (2000) found a lack of evidence for trichloroethylene-induced autoantibody production and systemic-lupus-erythematosus-like disease in Brown Norway rats when trichloroethylene was given by oral gavage 5 days a week for 6 weeks at 100-400 mg/kg.

Changes in Hematologic Parameters in Dogs

Hobara et al. (1984) found that acute inhalation exposure to trichloroethylene (200 and 500 ppm) or intravenous injection (50 mg/kg) in beagles produced a transient decrease in circulating leukocytes, most notably neutrophils that rebounded to near control concentrations after several hours.

HUMAN STUDIES

Following is a brief qualitative review of some human studies that have investigated trichloroethylene in relation to immunologic end points. It is provided to give a perspective on some of the important areas to be pursued as part of the risk assessment for trichloroethylene. A more thorough review of the epidemiologic studies in terms of methods, exposures, and results are necessary to fully characterize the immunologic hazards posed by trichloroethylene (see Chapter 2 for guidance on how this should be done).

Immunosuppression and Immunomodulation

Byers et al. (1988) reported on a human leukemia cluster putatively exposed to high concentrations of trichloroethylene and other solvents via contaminated drinking water. There were long-term alterations in peripheral blood T-cell subsets in the family members of those with leukemia, which suggests of an immunologic abnormality. There were increases in infections as well as an increased number of autoantibodies in this cohort. Lehman et al. (2002) found that children exposed in utero to volatile organic compounds, including trichloroethylene, had a shift toward TH_1 γ-interferon-producing T cells analyzed 6 hours after birth. These two studies suggest that immunologic changes may be seen in solvent- or trichloroethylene-exposed humans, although it has been difficult to quantify the exposures.

Autoimmunity

Numerous investigators have found an association between exposure to organic solvents, including trichloroethylene, and the human autoimmune diseases scleroderma (Saihan et al. 1978; Lockey et al. 1987; Waller, et al, 1994; Bovenzi et al. 1995, 2004; Nietert et al. 1998, 1999; Pandey and Takeuchi 1999; Pandey et al. 2001; Czirjak and Kumanovic 2002; Garabrandt et al. 2003; Pandey 2004) and Stevens-Johnson Syndrome (Pantucharoensri et al. 2004).
. The risk of scleroderma may be correlated with particular CYP2E1 and CYP2C19 polymorphisms (Povey et al. 2001), suggesting that trichloroethylene metabolism could be important in this disease. However, more research is needed to elucidate this possibility.

ISSUES FOR IMMUNOTOXICITY RISK ASSESSMENT

Extrapolation of Animal Data to Humans

A biologically plausible mechanism has been hypothesized for trichloroethylene-induced systemic autoimmunity and autoimmune hepatitis that involves the bioactivation of trichloroethylene to chloral in genetically susceptible mice. This mechanism might explain clonally restricted diseases, such as autoimmune-induced hepatitis, but does not explain polyclonal diseases. Chloral has been shown to bind to circulating proteins leading to an alteration in self that resulted in autoantibody formation and a chemically induced autoimmune syndrome. It is unclear whether this mechanism exists in humans, although it is notable that people with polymorphisms in CYP2E1 appear to have a higher risk of solvent-induced autoimmune disease. More studies are needed to incorporate aspects of innate and adaptive immune responses to study this and other proposed mechanisms of trichloroethylene-induced autoimmune diseases in humans.

Animal studies indicate that chronic exposure to trichloroethylene at moderate to high concentrations might have to potential to produce immunosuppression in animal models. However, there is no evidence to suggest that trichloroethylene is immunosuppressive in humans.

A potential biomarker for trichloroethylene exposure has been identified in mouse studies—namely, a chloral-protein adduct has been detected in tissues of trichloroethylene-treated mice (Griffin et al. 2000c). The usefulness of this marker in serum has not been demonstrated in humans.

Susceptibility

No general statements can be made about the susceptibility of rodent and non-rodent species to trichloroethylene compared with that in humans. It is important to evaluate potential mechanisms of immunotoxicity to determine whether those mechanisms are operative in humans. Pharmacokinetic modeling of specific metabolites of trichloroethylene is important to consider and apply to specific mechanisms that may be responsible for immunotoxicity.

Little is known about the genes that determine susceptibility to autoimmune and other immune diseases in humans. Although it is likely that environmental xenobiotics can act as triggers or exacerbants of autoimmune disease, there have not been adequate studies to make strong correlations. In addition, it is likely that multiple genes control susceptibility, some of which may play more important roles than others. There may be significant differences between rodent species and humans. It is important to explore genetic susceptibilities in human and animal models. Genes controlling metabolism and pharmacokinetic behavior of trichloroethylene likely are polymorphic and may influence effects on the immune system.

FINDINGS AND RECOMMENDATIONS

Laboratory results consistently show that some strains of mice are sensitive to autoimmune disease induction or exacerbation after exposure to trichloroethylene. The dose of

trichloroethylene required to produce effects depends on the route and length of exposure. It is difficult to extrapolate the results from genetically prone mice to humans, but these results suggest that humans with genetic susceptibilities may be at increased risk for autoimmune disease after exposure to by trichloroethylene. Animal data support the concept that trichloroethylene-mediated exacerbation of autoimmunity may be due to metabolism of trichloroethylene to chloral, which is a biologically plausible mechanism for humans.

Recommendations: More animal research is needed to clarify the metabolites and modes of action responsible for trichloroethylene-induced autoimmunity and immunosuppression. Epidemiology studies should further examine connective tissue diseases and other autoimmune diseases (including Stevens-Johnson Syndrome) or immunologic alterations (e.g., changes in T cell subsets, incidence of autoantibodies) in populations exposed to trichloroethylene.

Results from mouse studies suggest that choral forms protein adducts that lead to an alteration of self proteins and the production of autoantibodies. CYP2E1, a known human polymorphic enzyme, may play a role in the formation of these protein adducts (Griffin 2000c). Inhibition of CYP2E1 was found to decrease the incidence and severity of autoimmune diseases in mice. Therefore, genetic polymorphisms in CYP2E1 may play a role in exacerbating autoimmune disease.

Recommendation: Genetic polymorphisms that may play a role in the metabolism of trichloroethylene should be further examined to determine sensitivity factors and to characterize potentially sensitive populations.

9

Special Populations and Susceptibility

The goal of the U.S. Environmental Protection Agency's (EPA) public health risk assessments is to protect all potentially affected populations, including subpopulations on the basis of gender, nutritional status, genetic predisposition, and life stages (e.g., childhood, pregnancy, old age) that might be more susceptible to toxic effects or that are highly or disproportionately exposed (e.g., children, ethnic groups) (EPA 2004). Children have been identified as a special population to consider in risk assessment because their health risks can differ from those of adults as a result of their immature physiology, metabolism, and different levels of exposure (EPA 1996, 2005b). Certain childhood cancers have been associated with exposures to solvents, including trichloroethylene, which is briefly discussed in this chapter.

Data on differential impacts to sensitive populations are frequently limited or absent, and the process for consideration of sensitive groups is poorly defined. As EPA stated, there is "not a single or exact method for examining potential susceptibility and associated risk" (EPA 2004, p. 43). In its draft risk assessment on trichloroethylene, EPA (2001b) relied on the evaluation by Pastino et al. (2000), who correctly stated that measures of susceptibility have not been incorporated into human risk assessment methods. Several papers have since been published that are relevant to this issue, particularly to pediatric risk and genetic susceptibility. These papers as well as relevant older studies and information on gender- and disease-based susceptibility are reviewed below.

CHILDHOOD CANCER

A sizable number of published scientific studies are relevant to the issue of parental occupational exposure to trichloroethylene and childhood cancer. The studies generally involve parental occupational exposures based on case-control studies. The committee did not have the time or resources to analyze all the studies, so it relied on a review paper (Colt and Blair 1998) and some new studies to illustrate the issues important in estimating the public health risk of parental exposure to occupational trichloroethylene and risk conveyed to their children. Chapter 2 provides guidelines for conducting a more rigorous review of the epidemiologic data.

The effects of parental occupational exposure on the risk of childhood cancer have been studied epidemiologically for more than 25 years. During that time, in most countries the nature of industry has changed in two important ways: through materials usage and levels of exposure. Specifically, trichloroethylene has been largely phased out as an industrial solvent. It is important to keep this in mind when reviewing the studies and their chronology. Colt and Blair (1998) reviewed information on parental exposure to solvents and the risk of childhood cancer. They summarized results from 48 published papers, virtually all of which were case-control studies. A few later papers have not clarified whether a relation exists (Shu et al. 1999; Schuz et al. 2000; McKinney et al. 2003; Infante-Rivard et al. 2005).

All studies used the case-control approach and therefore relied on questionnaire information, raising the usual methodological issues of the reliability of identifying specific exposures, selection of controls, and recall bias. Most studies relied on information about occupation and industry to infer exposures rather than questioning subjects about exposure to specific chemicals. Ages of the children studied varied from study to study. Often, only the mother was interviewed and asked about both her and her husband's occupational history. It is very unlikely that women know about specific occupational exposures of their husbands, which is illustrated in two studies by Peters et al. (1981, 1985). In the first study, excess childhood brain cancer was associated with maternal and paternal exposure to chemicals, specifically to paint, and to work in the aerospace industry. An open-ended question addressed to the mother about specific chemicals to which either parent was exposed revealed little for the mothers and two mentions of trichloroethylene for the fathers. A follow-up study in which the fathers were interviewed revealed that five of them had exposure to trichloroethylene, whereas no control fathers did. Much like the community studies, the same occupations had other exposures to materials such as methylethylketone and other unspecified solvents. Following the approach of asking both parents about exposure to specific chemicals, Lowengart et al. (1987), in studying childhood leukemia, showed a risk associated with paternal exposure to chlorinated hydrocarbons (most of which was trichloroethylene). Greater use was associated with higher risk. The previously cited study (Shu et al. 1999), using similar techniques in a larger study population, failed to show a risk associated with paternal exposures to trichloroethylene but the study period was considerably later when trichloroethylene was less likely to be used and when exposures were likely lower.

Theoretically, there are several ways that risk can be conveyed from parent to child. When the mother is exposed, her child might be exposed in utero, through breast milk, from contaminated clothing of the mother, or from germinal effects. For paternal exposure, bringing contaminated clothing home is possible or direct germinal effects to males transmitted during reproduction are possible. The latter is the most likely for trichloroethylene, given supportive animal toxicologic data (see Chapter 5).

Early studies on paternal occupational exposures suggest that exposure to trichloroethylene conveys a risk of leukemia and brain cancer developing in the offspring. Later studies do not show that risk. Over the time period of these studies, trichloroethylene was phased out as an industrial solvent and exposures in work settings have generally been reduced. Thus, the occupational studies provide data that suggest a relationship between parental exposure to trichloroethylene and risk of childhood cancers. The difficulties of studying rare diseases and the inability to measure exposure objectively limit any certainty about causality.

DEVELOPMENTAL ISSUES

Fetal and Pediatric Risk Assessment Concepts

Chemical-specific toxicokinetic and mode-of-action information are essential for fetal and pediatric risk assessment. As stated by a previous committee of the National Research Council, complete data would include the following (NRC 2000, p. 3):

1. the chemical's toxicokinetics (i.e., its absorption, distribution, metabolism, and excretion) within the mother, fetus, and embryo;
2. the chemical's toxicodynamics (i.e., how the chemical or a metabolite derived from it interacts with specific molecular components of developmental processes in the embryo and fetus or with maternal or extraembryonic components of processes supporting development);
3. the consequences of those interactions on cellular or developmental processes (also part of toxicodynamics); and
4. the consequence of the altered process for a developmental outcome, namely, the generation of a defect.

A framework evaluating developmental risk is similar to adult risk assessment in that it includes exposure assessment, toxicity assessment, and risk characterization. Each of these items involves evaluation of multiple parameters (Figure 9-1).

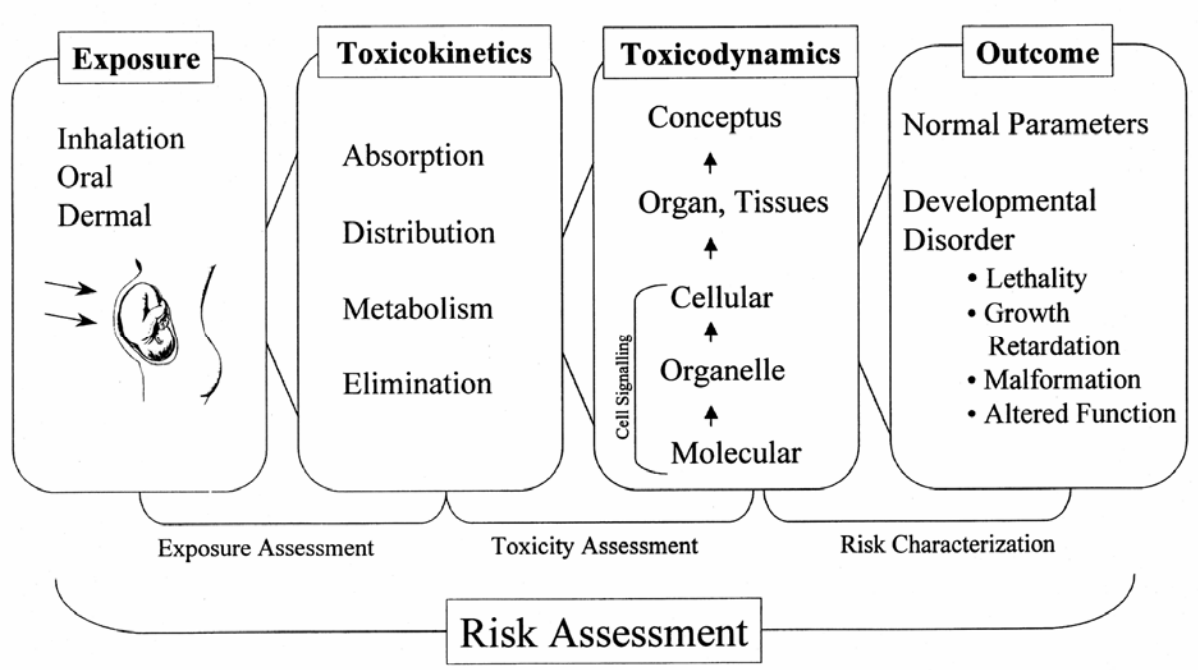

FIGURE 9-1 Overall framework to describe assessment of the effects of a toxicant on development.
Source: NRC 2000.

Tremendous advances have been made in recent years that facilitate the assembly of developmental toxicokinetic models. Such data include relevant ontogenic information about many of the phase I and phase II enzymes (Hines and McCarver 2002; McCarver and Hines 2002), which should be used for risk assessment (Dorne 2004). For the assessment of trichloroethylene, substantial data are available on toxicokinetics. Some, albeit less, information is available for items regarding developmental processes and their consequences (see Chapter 5). These advances, although still incomplete, can improve the precision of the risk assessment for trichloroethylene by narrowing uncertainty.

Approaches to Developmental Risk Assessment

Approaches defined under the new EPA guidelines for adult cancer risk assessment includes flexibility and the use of biologically based response models when appropriate (EPA 2005a). Similar flexibility and appropriate use of models are merited in evaluating toxicant risk for children under the ethical concept that children deserve, at a minimum, the same level of protection as adults. For any specific chemical, developmental susceptibility to toxicants could be addressed in several ways depending on the information available. These include the following:

1. Using developmental physiologically based pharmacokinetic (PBPK) modeling with parameter estimates appropriate for children: Such models can improve the development of relative-risk information for a specific xenobiotic exposure (Ginsberg et al. 2004a). The PBPK approach has already been used to evaluate pediatric drug therapy (Ginsberg et al. 2004b) and to assess adult risks from trichloroethylene (see Chapter 10); a similar effort should be applied for risk assessment in children. To facilitate such modeling, relevant data sets of developmental physiologic variables have been assembled and published (Haddad et al. 2001). A review of developmental pharmacokinetic modeling suggests that the 3.16-fold default uncertainty value commonly used for interindividual pharmacokinetic variability might be insufficient for some chemicals (Fawer et al. 1979). In contrast, Pelekis et al. (2001) developed a pediatric PBPK model for multiple volatile organic compounds using parameter estimates for a 10-kg child (1-2 years old) that suggests such children might not need additional protection. However, it is important to recognize that their model has not been validated with any in vivo developmental data; potentially this could be done with observational data. In addition, the model addresses only the parent compound and not the fractional metabolic clearance of any putative toxic metabolites; it is also a single-age model that does not reflect every period of childhood. For example, toddlers typically have enhanced clearance compared with younger and older children, as well as adults, because of enhanced relative liver and kidney size. Thus, although this is a good start in utilizing the PBPK approach, additional work should be done to ensure that the observation is robust and to assess the degree to which it applies across development. As stated by Ginsberg et al. (2004a), multiple age-appropriate physiologic models are needed. Because available information about the ontogeny of pathways involved in trichloroethylene disposition is substantial (see below), this approach would markedly decrease uncertainty about trichloroethylene exposures for the fetus and the child. However, similar to adults, this approach will not assess toxicodynamic differences and, importantly, will not address end points that might be unique to the fetus and the child. To be optimal, the information must be further

integrated using a serial approach that considers exposures serially from in utero to adulthood. Regardless of the limitations, this approach will significantly improve precision in risk estimates for children.

2. Using a developmental uncertainty factor: Such a factor could be added to the adult analysis to provide an arbitrary measure of added safety in consideration of a potential differential developmental susceptibility. This empiric approach has been used for risk assessment for childhood susceptibility for multiple chemicals. However, the approach should be used with decreasing frequency or the precision of the uncertainty factors should improve as more data become available. For trichloroethylene, insufficient toxicodynamic data necessitates using an uncertainty factor for toxicodynamic effects, but the uncertainty of increased susceptibility from exposure might be eliminated by using developmental PBPK modeling.

3. Establishing that children do not need greater protection than adults: If the variability present in adults is such that a sufficient margin of safety is present, an empiric decision could be made that no additional protection is needed for children. Generally, in risk assessment, this strategy is deemed appropriate when the variability is small and any estimate that ignores it will not be far from the truth. For developmental risk assessment, such a decision requires substantial justification, including detailed documentation of no added toxicodynamic risk during development. For trichloroethylene, as well as most other compounds, insufficient information is available to justify this approach.

Human Disposition of Trichloroethylene

EPA has clearly recognized the wide variability in trichloroethylene disposition, assigning a 50-fold variation, and also correctly recognized that this variability might contribute to susceptibility. Among adults, trichloroethylene disposition varies at least 7-fold, and perhaps as much as 50-fold (Lipscomb et al. 1997; Fisher et al. 1998). Variation in all aspects of trichloroethylene disposition—including absorption, distribution, metabolism, and excretion—affects the degree of biologic exposure to trichloroethylene and its metabolites (Astrand 1975). For example, trichloroethylene is poorly water soluble and highly fat soluble. After the same dose, trichloroethylene blood concentrations and urinary excretion of metabolites are expected to be greater in obese than in slim individuals (Sato et al. 1991). Similarly, the blood concentration of trichloroethylene and the total trichloroethylene body burden are expected to be higher in women than in men (Sato et al. 1991). Physical exertion during exposure to trichloroethylene, which is associated with increased pulmonary ventilation and cardiac output, is associated with increased adsorption, blood concentrations, and metabolite excretion (Astrand 1975).

Trichloroethylene is metabolized to chloral, which spontaneously hydrates to form chloral hydrate (Byington and Leibman 1965). This initial metabolic step, which might involve the transient formation of an intermediate epoxide (Miller and Guengerich 1982), is rate limiting and is catalyzed predominantly by the cytochrome P-450 enzyme CYP2E1 (Ikeda et al. 1980; Nakajima et al. 1992a; Lipscomb et al. 1997). Of multiple cytochrome P-450 enzymes tested in vitro, trichloroethylene metabolism to chloral hydrate correlated only with immunologically detected CYP2E1, and chloral hydrate formation significantly correlated with the oxidation of known CYP2E1 substrates (Lipscomb et al. 1997). The metabolism of chloral hydrate depends on two forward pathways (oxidation to trichloroacetic acid and reduction to trichloroethanol) and one back reaction (forming chloral hydrate from trichloroethanol). The reduction of chloral

hydrate to trichloroethanol is catalyzed by alcohol dehydrogenase (Friedman and Cooper 1960) and is NADH dependent, whereas the oxidation of chloral hydrate to trichloroacetic acid is mediated by aldehyde dehydrogenase (Cooper and Friedman 1958). The back reaction forming chloral hydrate from trichloroethanol appears to be catalyzed by CYP2E1 (Barton et al. 1996).

Trichloroethanol is also conjugated with glucuronate and the resulting water-soluble conjugate is excreted in the urine. Whether dichloroacetic acid is formed is controversial, with some investigators (Henderson et al. 1997) but not others documenting its presence in very low amounts in the blood of children given chloral hydrate for therapeutic indications. Brashear et al. (1997) found dichloroacetic acid at the limits of detection for mass spectrometry in adults exposed to trichloroethylene at 100 parts per million. Others have suggested that the presence of dichloroacetic acid is an analytic artifact and that dichloroacetic acid, if formed, is rapidly removed, precluding its measurement (Merdink et al. 1998). Further, no mechanism of dichloroacetic acid formation in humans has been described. The alternative to the initial CYP2E1-mediated trichloroethylene oxidation to chloral hydrate is direct conjugation of trichloroethylene with glutathione. This pathway accounts for less than 1% of the disposition of trichloroethylene and ultimately leads to the formation of cysteine conjugates or mercapturates (Green et al. 1997a). Although quantitatively a minor pathway, these metabolites have been associated with renal carcinogenesis and therefore are important in risk assessment (see Chapter 3).

Although intersubject differences in adsorption and distribution occur among adults, most of the variation in trichloroethylene disposition is secondary to differences in metabolism. In microsomal preparations from 23 human livers, CYP2E1-mediated trichloroethylene intrinsic clearance (V_{max}/K_m) to chloral hydrate varied about 6-fold (Lipscomb et al. 1997). Among healthy volunteers exposed to ambient trichloroethylene, the urinary excretion of trichloroacetic acid and trichloroethanol varied 6- to 7-fold (Fisher et al. 1998), whereas trichloroacetic acid formation after dosing with chloral hydrate varied almost 10-fold, with 5% to 47% of the dose excreted as urinary trichloroacetic acid (Marshall and Owens 1954). In contrast to this striking between-subject variability, within subject variability appeared to be less than 2-fold.

Developmental Toxicokinetic Information

Improved analysis of trichloroethylene developmental toxicokinetics is important because recent population studies suggest that 3% to 7% of children have measurable amounts of trichloroethylene in their blood (Sexton et al. 2005). Although less is known about trichloroethylene disposition in children than in adults, substantial information is available that merits consideration in risk analysis. This includes information about the developmental profiles of CYP2E1, alcohol dehydrogenase, and aldehyde dehydrogenase, as well as studies of the disposition of chloral hydrate in children. The developmental profile of CYP2E1, the enzyme responsible for the rate-limiting step, has been well characterized across fetal and pediatric age groups (Johnsrud et al. 2003). Further, substantial pharmacokinetic information is known about the disposition of the major proximate metabolite, chloral hydrate, a sedative commonly used in children. Dichloroacetic acid, which may or may not be a metabolite in humans, is used therapeutically in the relatively rare disorder congenital lactic acidosis, and some pharmacokinetic data are available in adults and children, so that age-related comparisons are possible.

Important Physiologic Changes

Many physiologic changes directly affect developmental toxicokinetics, whereas others constitute toxicodynamic differences or additional toxicodynamic targets not present in adults. The biologically relevant internal dose of trichloroethylene as well as that of its metabolites likely is altered by multiple physiologic developmental changes. These include not only overall growth but also changes in body composition, relative organ size, and hormonal changes. Of particular importance to trichloroethylene and other compounds that deposit into fat is the doubling of body fat during early infancy with a concomitant fall in the amount of total body water. The liver and kidney, the predominant organs of overall toxicant activation and deactivation, are severalfold larger relative to body weight in children than in adults; this effect is greatest among toddlers (Maxwell 1984). Growth hormone concentrations increase during the newborn period and again with puberty (Quattrin et al. 1990; Rose et al. 1991; Main et al. 1994). Such growth hormone changes are associated with differences in drug disposition and drug-metabolizing enzyme expression (Redmond et al. 1978; Lambert et al. 1986; Butler et al. 1989). Growth hormone changes are associated with alterations in developmental gene expression mediated by many pathways, including the early response genes c-*fos* and c-*jun*, and HNF-6, a hepatic transcription factor that activates a network involved in cytochrome P-450 and plasma protein gene regulation (Rastegar et al. 2000). In addition to the differences in disposition that occur from growth and development, the fact that growth and development are occurring provides additional targets for derangement that are not present in adults. Thus, there are striking physiologic differences in children that affect xenobiotic disposition and likely alter their risk.

Developmental differences have been demonstrated in every aspect of pharmacokinetics; including absorption, distribution, metabolism, and excretion, using various therapeutic agents (see review by McCarver 2004). The impact of these differences on toxicokinetics has been less well evaluated. However, as stated above, in vitro and in vivo data have been generated that might facilitate PBPK modeling of trichloroethylene exposure in children. Oral and percutaneous absorption differences have been documented with many therapeutic agents (Heimann 1981; West et al. 1981; Rutter 1987; Barrett and Rutter 1994). Both routes are relevant for trichloroethylene exposure. Neither has been studied specifically for trichloroethylene; however, information generated for other compounds could be used to model the expected differences from these factors. Developmental variation in oral absorption is most marked in infancy and is due to differences in gastric pH, gastric emptying, pancreatic enzymes, and first-pass metabolism in the stomach, small intestine, and liver. Developmental differences in percutaneous absorption are due to differences in skin thickness, vascularization, and hydration. Distribution differs with age because of changes in body composition, protein, and tissue binding (Heimann 1981; Fisher et al. 1982; Nau et al. 1983; Lerman et al. 1989). An important distribution issue for the fetus is the ability of compounds to redistribute from amniotic fluid back to the fetus. In a rodent model, trichloroacetic acid was found to cycle from the fetus into the amniotic fluid and back into the fetus (Ghantous et al. 1986). Thus, amniotic fluid could act as a reservoir. Tissue drug binding, a more direct marker of the pharmacokinetic-pharmacodynamic interface than plasma values, might also be age dependent for some compounds (Park et al. 1982). Although the distribution of drugs across membranes recently has been shown to be influenced by a growing number of drug transporters, study of their ontogeny has just begun. It appears unlikely that a small, lipophilic, highly diffusible compound such as

trichloroethylene would require a transporter, but this issue has not been addressed. In summary, although these processes are complex, sufficient information exists to support exploration of PBPK modeling for trichloroethylene pediatric exposure.

Ontogeny of Human Enzymes Involved in Trichloroethylene Disposition

The use of generalities about the direction and extent of differences in metabolism in children compared with adults in risk assessment cannot be supported by currently available data. The developmental expression pattern varies by enzyme (Hines and McCarver 2002; McCarver and Hines 2002). Moreover, human and animal data are likely to differ, and such interspecies differences could be substantive. In contrast to rodents, human CYP isoform expression occurs relatively early, generally before birth or within the first several months of life (Hines and McCarver 2002). CYP2E1, the enzyme responsible for the rate-limiting step in trichloroethylene disposition, is expressed by week 8 of gestation in human fetal cephalic tissue at greater concentrations than in corresponding human fetal hepatic tissue (Brzezinski et al. 1999). In humans, hepatic expression occurs as early as the second trimester and rapidly increases after birth, with adult expression levels being reached by 3 months (Carpenter et al. 1996; Johnsrud et al. 2003). In contrast, rodent hepatic CYP2E1 expression begins postnatally (Keeter et al. 1990). Importantly, human CYP2E1 developmental expression data are sufficiently complete to support developmental PBPK modeling.

Differences in human alcohol dehydrogenase might be of equal or greater relevance to human trichloroethylene risk assessment compared with CYP2E1. Although CYP2E1 is the rate-limiting enzyme, the halogenated acetic acids trichloroacetic acid and dichloroacetic acid have been suggested as the proximate putative teratogenic species (Johnson et al. 1998a) as well as, perhaps, proximate nonrenal carcinogens (Herren-Freund et al. 1987; DeAngelo et al. 1989). As such, variability in alcohol dehydrogenase, as well as aldehyde dehydrogenase variability (discussed below), would influence the amount of chloral hydrate that is diverted to trichloroacetic acid, one of the putative toxicants. If alcohol-dehydrogenase-mediated conversion to trichloroethanol, a theoretically less toxic metabolite, is limited from immaturity or genetic factors, then more chloral hydrate would be available for conversion to trichloroacetic acid. However, this has not been directly confirmed in humans. Human alcohol dehydrogenase is a superfamily consisting of at least five classes encoded at seven genetic loci. Class I alcohol dehydrogenase includes three isoforms (α, β, and γ), and dimers of this class are the most effective at metabolizing ethanol to acetaldehyde. Thus, these isoforms have been well studied with ethanol (see below). In contrast, the relative capability of the various alcohol dehydrogenase isoforms in reducing chloral hydrate to trichloroethanol has not been published. As a general rule, for class I alcohol dehydrogenase, alcohols with bulky substituents are better substrates than ethanol. The ontogeny of alcohol dehydrogenase was described more than 30 years ago in seminal work by Smith et al. (1971), which was replicated and expanded by Estonius et al. (1996). In late gestation, fetal alcohol dehydrogenase activity is about 25% that of adult activity (Pikkarainen and Raiha 1967). Although expression is greatest in liver, class I alcohol dehydrogenase transcripts are widely distributed in all organs except fetal and adult brain, adult kidney, and placenta. In first-trimester human fetal liver samples, alcohol dehydrogenase α, encoded at *ADH1A*, is the only detectable class I isoform. Beginning in the second trimester and continuing into the early third trimester, hepatic alcohol dehydrogenases α,

β, and γ (encoded by *ADH1A*, *ADH1B*, and *ADH1C*, respectively) are all present, but alcohol dehydrogenases α and β predominate. By the late third trimester, human hepatic *ADH1C* expression has increased markedly, but *ADH1B* expression still predominates. In adult liver, *ADH1A* expression is not detected, and expression of *ADH1B* and *ADH1C* is equivalent. In lung, only *ADH1B* expression is detected, and it is similar in adult and fetal samples (Estonius et al. 1996). Alcohol dehydrogenase class III is expressed in virtually all tissues, including in the fetus; expression in fetal brain appears to be somewhat greater than in adult brain.

Aldehyde dehydrogenase is a superfamily of $NAD(P)^+$-dependent enzymes whose characteristics and substrate specificity vary (Vasiliou et al. 2004). The aldehyde dehydrogenase isoforms are encoded at 17 genetic loci (at least), and the various forms are highly expressed in the microsomal, mitochondrial, and soluble fractions of the liver as well as at lower levels in other tissues (Koivula 1975). Which aldehyde dehydrogenase isoforms are capable of and most efficient at metabolizing chloral hydrate to trichloroacetic acid is unknown but needs to be determined. *ALDH1A1*, *ALDH1A2*, *ALDH1A3*, and *ALDH8A1* are involved in the oxidation of retinaldehyde to retinoic acid, a critical factor in many developmental processes and signaling events. *ALDH1A1*, *ALDH1B1*, and *ALDH2* are all involved in the oxidation of acetaldehyde, the proximate metabolite of ethanol. In addition, *ALDH1L1* hydrolyzes 10-formyltetrahydrolate to tetrahydrofolate, another reaction critical to development (Krupenko et al. 1997). Multiple forms of aldehyde dehydrogenase participate in the metabolism of 4-hydroxynonenal and malondialdehyde, two predominant products of human lipid peroxidation, and others in the formation of glutamate and in γ-aminobutyric acid metabolism. Treating rats with trichloroethylene depressed aldehyde dehydrogenase activity for short-chain aliphatic aldehydes in the mitochondrial and cytosolic fractions but not in the microsomal fraction (Wang et al. 1999), and this activity appears to be due to chloral hydrate [median inhibitory concentration = 8 μM] (Wang et al. 1999; Poon et al. 2002). Thus, human aldehyde dehydrogenase theoretically is an important interaction point between trichloroethylene and multiple xenobiotics as well as multiple physiologic mechanisms. As such, more information on its role in trichloroethylene and chloral hydrate metabolism is needed.

Glutathione *S*-transferases (GSTs) are members of a family of enzymes that conjugate glutathione to electrophilic compounds. However, as noted above, glutathione conjugation represents the initial enzyme in the toxification pathway associated with renal carcinogenesis. The enzyme consists of two homodimeric subunits from one of five GST subunit classes (alpha, beta, mu, pi, theta, and zeta), and each enzyme is designated with a letter indicating its class membership (A, B, M, P, T, and Z, respectively). Which GST is most efficient in conjugating trichloroethylene is unknown. GST ontogeny was recently reviewed (McCarver and Hines 2002; Ginsberg et al. 2004a). Briefly, the hepatic alpha isoforms, *GSTA1* and *GSTA2*, are detected in the early fetal period and reach adult levels in the first 1-2 years of life (Strange et al. 1989). Fetal hepatic isoform mu expression is about 22% of adult expression and increases about 5-fold shortly after birth to adult levels (Strange et al. 1989). Isoform pi is the predominant class in early hepatic development, being expressed in the human fetus and young infant at levels that exceed adult concentrations by 500- and 200-fold, respectively (Strange et al. 1989). *GSTA1* and *GSTA2* are also expressed in the fetal kidney and renal expression increases in the first two postnatal years, whereas *GSTM* is lower in postnatal than in fetal samples (Beckett et al. 1990). *GSTP1* has also been documented in early fetal renal collecting ducts (van Lieshout et al. 1998). Pulmonary developmental expression has also been evaluated with immunohistochemical and radioimmunoassays (Hiley et al. 1989). *GSTP1* is expressed at high concentrations in early fetal

pulmonary ductal columnar cells, but this expression decreases with gestation. In contrast, pulmonary expression of *GSTM*, *GSTA1*, and *GSTA2* are relatively low, but consistent, across gestation.

Chloral Hydrate and Dichloroacetic Acid Studies in Children

The trichloroethylene metabolite chloral hydrate has been used as a sedative for more than 150 years and is prescribed frequently for children, usually as a single dose for procedural sedation. Adults are typically given 1 g, whereas children are given 50-75 mg/kg up to the adult dose. The sedative effect is believed to be due to the metabolite trichloroethanol (Marshall and Owens 1954), although this assumption has been controversial (Mayers et al. 1992). Concern about chloral hydrate use in children includes its competition with bilirubin glucuronidation in the newborn period (Lambert et al. 1990) and the increased risk of arrhythmias, particularly among patients with congenital heart disease (Hirsch and Zauder 1986).

Concern about the possible carcinogenic potential of chloral hydrate in children (Steinberg 1993) resulted in a series of developmental studies by the National Toxicology Program (NTP 2002a,b). In a 2-year study, B6C3F_1 neonatal female mice under multiple chloral hydrate regimens, including groups given single or multiple doses for 2 years (both ranging from 0 to 100 mg/kg of body weight), the results were considered equivocal for carcinogenesis (NTP 2002a). The only positive result was an increased incidence of pituitary gland pars distalis adenomas at the highest dose (100 mg/kg for 24 months) without evidence of a dose response. Further, the incidence of pituitary adenomas in the high-dose group was similar to historical but not concurrent controls. No single-dose regimen was associated with carcinogenesis. In the 2-year B6C3F_1 male mouse study, increased incidence of hepatocellular adenoma or carcinoma (combined variable) was observed in mice fed ad libitum, and increased incidence of hepatocellular carcinoma was observed in dietary-controlled mice, which was associated with peroxisome proliferation (NTP 2002b). The concern over hepatocellular carcinogenesis was limited by the recognition that humans exhibited very weak liver peroxisome proliferative responses (Waxman 1999).

Although chloral hydrate is used clinically, the number of pharmacokinetic studies in children of various ages is limited. Critically ill infants and children (n = 22, ranging from 31 weeks postconceptional age to 13.6 years) participated in a pharmacokinetic study of chloral hydrate (Mayers et al. 1991). Patients were divided into three age groups, but it is unclear how many subjects were in each group. After a standard sedative oral dose (50 mg/kg, presumably to a maximum adult dose of 1 g), mean peak chloral hydrate plasma concentrations ranged from 3.89 ± 2.87 mg/L among toddlers and older children to 6.23 ± 2.28 mg/L among full-term infants to 8.01 ± 5.12 mg/mL among preterm newborns. After an initial rapid distribution phase, chloral hydrate terminal elimination occurred more slowly with a half-life of 1 to 10 hours. Apparent oral chloral hydrate clearance was about 5 L/hour/kg and did not differ by age. Trichloroethanol peak concentrations ranged from about 27 to 36 mg/L. Older children had a shorter trichloroethanol half-life of about 10 hours, whereas preterm and term infant groups had half-lives of about 40 and 28 hours, respectively. Because trichloroethylene is metabolized by glucuronidation, a pathway known to increase during the first few months of life, the observation of age-dependent trichloroethanol elimination was not surprising. Trichloroacetic acid increased to significant concentrations (10-20 mg/L) during the 7-day study and did not decline, so a half-

life could be determined only in one patient. The authors noted that the dose of metric area under the curve for trichloroacetic acid during the first 24 hours was greater among older children. They speculated that an inability of infants to form the oxidative metabolite trichloroacetic acid was responsible, which suggests that aldehyde dehydrogenase was immature in these subjects. Unfortunately, the study did not include urinary metabolite data, which are necessary for determining the relative conversion to trichloroethanol and to trichloroacetic acid. Henderson et al. (1997) described chloral hydrate kinetics in three children treated with chloral hydrate (50 mg/kg) alone; with chloral hydrate (50 mg/kg) 15 minutes before a dose of [^{13}C]dichloroacetic acid, or with two chloral hydrate doses before and after a dose of [^{13}C]dichloroacetic acid. Details of the gas chromatography-mass spectrometry assay including linearity and reproducibility were not given. Chloral hydrate peak values were not given. The peak concentration of trichloroethylene was 115 mg/mL at 25 minutes. Peak values for dichloroacetic acid (22 mg/mL) and trichloroacetic acid (65 mg/mL) occurred much later, at 7.5 and 11.5 hours, respectively. Importantly, the time course of trichloroacetic acid does not match that in other reports. Henderson et al. (1997) further reported coadministration of dichloroacetic acid with chloral hydrate prolonged the half-life of dichloroacetic acid, suggesting that chloral hydrate inhibits the metabolism of dichloroacetic acid. The pharmacokinetics of dichloroacetic acid have been determined in a few studies (Fox et al. 1996; Barshop et al. 2004), one of which included mostly children (Barshop et al. 2004). Among healthy adult volunteers (Curry et al. 1991), oral and intravenous dichloroacetic acid bioavailability were similar, and no gender difference was observed. The apparent terminal half-life was noted to increase with chronic dosing by more than 20-fold in healthy adults (Curry et al. 1985, 1991) and by more than 10-fold in lactic acidosis patients (Barshop et al. 2004). Among adults treated for lactic acidosis with single doses of dichloroacetic acid varying from 30 to 100 mg/kg, dichloroacetic acid elimination was zero order at concentrations above 80-120 μg/mL and first order at lower concentrations with a terminal half-life of about 1.2 hours (Fox et al. 1996). The typical maximum concentration in both adult and pediatric lactic acidosis patients is 120-160 μg/mL after 50 mg/kg intravenously (Fox et al. 1996; Barshop et al. 2004). Among 37 patients, including 31 children (ages 7 months to 17.8 years) treated with either single doses (50 mg/kg/day, n = 6) or repeated doses (variable doses, n = 31) for congenital lactic acidosis, the terminal half-lives were 86 minutes and 11 hours, respectively (Barshop et al. 2004). Although studies in healthy adult volunteers are relevant to the general population, metabolic studies in children and adults with lactic acidosis might not be relevant as the underlying acid-base abnormalities could alter enzymatic activity. Cord blood samples from 52 women given chloral hydrate during labor indicated that chloral hydrate crosses the human placenta (Bernstine et al. 1954). Chloral hydrate, trichloroethanol, and trichloroacetic acid were the same or higher in 50%, 63%, and 57% of cord blood samples, respectively, compared with paired maternal blood samples. Concentrations in amniotic fluid samples were described as matching fetal samples. In contrast to information for chloral hydrate, data on the in vivo human disposition of low concentrations of trichloroethylene in pregnant women have not been reported but are needed. The human ontogeny of both dichloroacetic acid and chloral hydrate metabolism in vitro, as well as chloral hydrate metabolism in vivo, merits further definitive study so that the data could be used for improved PBPK modeling.

GENETIC SUSCEPTIBILITY

Currently, 114 human CYP2E1 single nucleotide polymorphisms have been reported to the National Center for Biotechnology Information database dbSNP. Of these, 65 have been validated. Of the validated single nucleotide polymorphisms, 40 occur in introns, 3 occur in the mRNA untranslated region, and 22 occur in the coding sequence or in the upstream sequence. For many single nucleotide polymorphisms, the functional significance, if any, is unknown. Three allelic variants, *CYP2E1*2*, **3*, and **4* have been defined, each of which contains a single nucleotide polymorphisms leading to an amino acid change. However, two of these, *CYP2E1*3*, consisting of a guanine to adenine substitution at nucleotide (10023 G>A) (leading to a valine to isolencine change at amino acid 389 [V389I]), and *CYP2E1*4*, consisting of 4768 G>A (leading to V179I), were associated with normal in vitro CYP2E1 activity (Hu et al. 1997; Fairbrother et al. 1998). In contrast, 1168 G>A causes an arginine to histidine substitution (R76H), which was associated with about a two-thirds decrease in both enzyme expression and activity in vitro (Hu et al. 1997). However, the variant is rare, occurring in about 1% of Asian populations and not detected among Europeans. Another single nucleotide polymorphism, adenine to thymine at nucleotide 11112 (11112 A>T), is associated with a histidine to leucine substitution (H457L), but has not been given a haplotype designation, and its functional significance has not been reported in vitro or in vivo (Solus et al. 2004). Two haplotypes, *CYP2E1*1B* and *CYP2E1*1D*, contain genetic polymorphisms in the upstream regulatory region. *CYP2E1*1D* has been shown to be associated with increased CYP2E1 activity in vivo among individuals who are obese or who consume ethanol (McCarver et al. 1998). This insertion of 96 base pairs in the CYP2E1 regulatory regions occurs in about 25% of African Americans and a smaller percentage of Caucasians (McCarver et al. 1998); it represents the only known frequent, functional human CYP2E1 polymorphism described to date.

As noted above, it is unknown which human alcohol dehydrogenase isoform is most efficient at oxidizing chloral to trichloroethanol. The loci encoding two of the three class I alcohol dehydrogenase isoforms, *ADH1B* and *ADH1C*, exhibit functional genetic polymorphisms. Two *ADH1B* variants, *ADH1B*2* and **3*, have been associated with decreased susceptibility to fetal alcohol spectrum disorders (McCarver et al. 1997; Viljoen et al. 2001; Das et al. 2004; Warren and Li 2005), presumably from increased ethanol elimination by the enzymes these variants encode (Thomasson et al. 1995; Neumark et al. 2004). Similarly, alcohol dehydrogenase genetic polymorphisms have been associated with differences in the risk for alcohol dependence (Thomasson et al. 1991) and cancer in some studies (Yokoyama et al. 2001; Yang et al. 2002; Coutelle et al. 2004) but not others (Olshan et al. 2001). The alcohol dehydrogenase variants are relatively common, occurring at allelic frequencies of 20% to 50%, depending on the ethnic group (Warren and Li 2005). Thus, it is plausible that the same alcohol dehydrogenase polymorphisms could be important factors for trichloroethylene teratogenic and carcinogenic susceptibility; studies directly addressing this hypothesis are needed.

Twenty-two aldehyde dehydrogenase genetic variants have been described, of which at least 11 appear to be functional (Vasiliou et al. 2004; www.aldh.org). Among the most studied variants is *ALDH2*2*, which encodes a low-activity variant of mitochondrial aldehyde dehydrogenase and occurs in about 30% of individuals of Asian descent (Thomasson et al. 1991). This genetic variant is associated with a lower frequency of alcohol dependence and inability to oxidize acetaldehyde, yielding a flushing reaction during ethanol consumption (Itoh et al. 1997). Monte-Carlo analyses of the variability factors of other somewhat similar

compounds, such as ethanol and toluene, suggest that the default pharmacokinetic uncertainty factor is not sufficient to account for the *ALDH2*2* polymorphism (Ginsberg et al. 2002). The impact of aldehyde dehydrogenase variation on trichloroethylene disposition is unknown but is critical information for integrating genetic information into risk assessment.

GSTs are a family of phase II enzymes involved in the metabolism of many xenobiotics (Mannervik et al. 1992). Mammalian GSTs belong to three families of proteins that are expressed in cytosol, mitochondrial, and microsomal cellular fractions (Hayes et al. 2005). GSTs are catalytically active as hetero- or homodimers. These proteins are expressed in most human tissues, although numerous isoforms and subtypes are differentially expressed in cells and tissues (Strange et al. 1991). At least 16 cytosolic GSTs have been identified. These enzymes are named based on their amino acid sequences and immunologic characteristics (Board 1981; Mannervik 1985; Board et al. 1990). In general, mammalian cytosolic GSTs are divided into seven classes, which have been named alpha, mu, pi, sigma, theta, zeta, and omega. Many of the isoforms have known polymorphisms (Hayes et al. 2005). Examples include *GSTM1*A* (associated with normal protein levels and activity), *GST1*B* (associated with low protein levels), *GSTM1*0* (a gene deletion that leads to a null phenotype), and *GSTM1*1 × 2* (gene duplication). *GSTM1*0* is seen in more than 50% of some populations (Board et al. 1990). The *GSTT* family has at least three known polymorphisms (Strange et al. 1984). Various classes of GSTs, especially their null mutants, have been associated with cancer (Strange et al. 1991; van Poppel et al. 1992).

Whereas GSTs are generally considered to be important in the deactivation of electrophiles and oxidants, there are a few examples of bioactivation reactions. In these cases, the glutathione conjugate of a xenobiotic metabolite is more toxic than the xenobiotic or its metabolite alone. Such is the case for the glutathione conjugate of trichloroethylene known as *S*-1,2-dichlorovinyl-L-glutathione, which forms *S*-1,2-dichlorovinyl-L-cysteine in the presence of β-lyase (Dekant 1986). *S*-1,2-Dichlorovinyl-L-cysteine has been associated with kidney cancer (see Chapter 3), particularly in humans with a mutation in the von Hippel-Lindau gene. Populations with increased *GSTT1* activity and a *GSTM1* null allele have been found to be at particular risk for renal cell cancer (Bruning et al. 1997b).

Thus, depending on the specific GST isoform involved, a polymorphism in these enzymes can be expected to increase or decrease cancer risk. Predicting the human cancer or non-cancer risks in humans depends on the specific gene and polymorphism expressed. Data for trichloroethylene are incomplete; which GST isoforms are most efficient in trichloroethylene disposition is unknown.

ACQUIRED STATES WITH POSSIBLE ALTERED SUSCEPTIBILITY

Multiple conditions, including ethanol ingestion, exposure to other solvents, fasting or starvation, obesity and diabetes, and consumption of some popular dietary items, such as green tea, have been shown to induce CYP2E1 (McCarver et al. 1998; Lieber 2004; Liangpunsakul et al. 2005; Yang and Raner 2005). Many conditions associated with CYP2E1 induction are quite common and therefore likely to occur concomitantly with trichloroethylene exposure. Alcoholism affects about 14 million Americans annually; it is estimated that about half of Americans over the age of 12, or about 110 million Americans, consume ethanol (SAMHSA 2003). Thus, a clear understanding of the interactions between trichloroethylene and ethanol and

the effect on the trichloroethylene risk assessment is needed. The interaction between ethanol and trichloroethylene is complex and includes alterations in trichloroethylene kinetics and dynamics as well as those of its metabolites (for full discussion, see review by Pastino et al. [2000] and Chapter 10). Chronic heavy drinkers are likely to have enhanced trichloroethylene metabolism due to CYP2E1 induction. However, this is likely to be most relevant at high concentrations of trichloroethylene at which saturation would occur in the absence of enzyme induction. Chronic heavy drinkers who have progressed to cirrhotic liver damage could have decreased trichloroethylene metabolism. Individuals who have not recently consumed sufficient ethanol to induce CYP2E1 but who have acute, relatively concomitant exposure have decreased trichloroethylene metabolism from competitive inhibition (Muller et al. 1975). In addition to the effect on disposition, simultaneous exposure to ethanol and chloral hydrate increases the sedative effects, perhaps from the ethanol-mediated shift in choral hydrate metabolism from oxidation to trichloroacetic acid to reduction to trichloroethanol (Watanabe et al. 1998). Obesity and diabetes induce CYP2E1 and both occur in millions of Americans (Harris 1995; Flegal et al. 2002). Although the impact of these common diseases would be expected to be similar to that from ethanol- or other solvent-mediated CYP2E1 induction, the relationships might be more complex. For example, increased body fat content affects trichloroethylene distribution so that urinary excretion rates are estimated to be greater in thin men than in obese men (Sato 1993). This effect might offset any enhanced CYP2E1-mediated metabolism in obesity. Notably, trichloroethylene-induced hepatotoxicity was not enhanced in a chemically induced rodent model of diabetes (Hanasono et al. 1975). Thus, direct study of the impact of these common states on trichloroethylene disposition and risk merits direct investigation.

GENDER

Simulated models suggest that, after the same dose of trichloroethylene, women have a slightly greater total body burden of trichloroethylene and its metabolites than men, reflected in a larger amount of total urinary metabolites (Sato et al. 1991). However, the size of this gender difference was relatively small (blood trichloroethylene concentrations 30% higher after 16 hours), and it was due to increased body fat content in women. In 16 volunteers (8 of each gender), equivalent ambient trichloroethylene exposure (100 parts per million for 4 hours) resulted in about 3.4-fold higher maximal concentrations and area-under-the-time-concentration curves of *S*-1,2-dichlorovinyl-L-glutathione in men than in women (Lash et al. 1999). This difference is intriguing because male rats were found to be efficient at trichloroethylene glutathione conjugation and male rat tubular cells were more susceptible to acute toxicity induced by *S*-1,2-dichlorovinyl-L-glutathione and *S*-1,2-dichlorovinyl-L-cysteine (Lash et al. 2001b). In contrast, one human case-control study of renal carcinogenesis showed an increased susceptibility for women (Dosemeci et al. 1999).

HUMAN VARIABILITY AND THE USE OF UNCERTAINTY FACTORS

Uncertainty factors are applied to risk estimates to account for variability in human populations as well as other factors. Following is a synopsis of EPA's use of uncertainty factors in its draft health risk assessment for trichloroethylene.

The oral reference dose for trichloroethylene non-cancer effects was calculated as 3×10^{-4} mg/kg-day, based on subchronic studies of rats and mice that showed effects at 1mg/kg-day. The uncertainty factors included the following:

- A 50-fold uncertainty factor to account for differences between average and sensitive humans. This value was calculated by multiplying the value chosen for human pharmacokinetic variability (set at 15-20, see discussion below) by a $10^{1/2}$-fold[a] pharmacodynamic variation, which is the EPA default value.
- A $10^{1/2}$-fold default uncertainty factor for animal-to-human pharmacodynamic uncertainty. The previous value (15-20) was considered to account for animal-to-human pharmacokinetic uncertainty.
- A $10^{1/2}$-fold uncertainty for using subchronic instead of lifetime studies. EPA states that duration-response trends are not evident in animal studies, but some human studies indicate that prolonged exposure to trichloroethylene can increase the severity of effects. Therefore, the partial $10^{1/2}$-fold uncertainty was used "until duration-response relationships are better characterized in humans."
- A $10^{1/2}$-fold uncertainty factor for extrapolation from a lowest-observed-adverse-effect level (LOAEL) to a no-observed-adverse-effect level (NOAEL) because adverse effects were observed at the 1 mg/kg-day point of departure. EPA stated that the standard 10-fold default was not used because "1 mg/kg-day appears to be at the boundary where effects can begin to be observed."
- A $10^{1/2}$-fold uncertainty factor to reflect background exposures to trichloroethylene and its metabolites to address cumulative risks.

In total, this generates a 5,000-fold uncertainty factor. Ultimately, however, a factor of 3,000 was used as the divisor because it is the largest divisor used by EPA in the presence of substantial uncertainty (EPA 2001b).

The trichloroethylene inhalation concentration of 4×10^{-2} mg/m^3 was based on a subchronic exposure of 38 mg/m^3 showing adverse effects on the central nervous system in human occupational studies. The uncertainty factors included the following:

- A 10-fold default uncertainty factor for human variation,
- A 10-fold default uncertainty factor for using subchronic instead of lifetime studies, and
- A 10-fold default uncertainty factor for extrapolation from a LOAEL to a NOAEL uncertainty because the central nervous system and endocrine effects were LOAELs observed in occupational studies.

For cancer end points, EPA chose to use a range of slope factors in view of risk factors that can modify the effects of trichloroethylene in different populations. Of the cancer studies evaluated (including rodent and human studies), the highest and lowest cancer slope factors were not used and the range of the remaining studies was maintained. According to EPA "these

[a] $10^{1/2}$ (the square root of 10, approximately equal to 3) represents half a factor of 10 on a logarithmic scale. In this case, the $10^{1/2}$ factor was the default for the pharmacodynamic variation. In other instances (e.g., LOAEL to NOAEL extrapolation), the use of this lesser factor is supported by qualitative information presented in EPA's discussion of the uncertainty factors.

remaining estimates constitute a middle range of risk estimates where confidence is greatest" (EPA 2001b). Recognizing differences in human responses and the potential for sensitive populations, EPA further states that "a single risk value is not appropriate to describe the differential effects of [trichloroethylene]" and "alternative slope factors have not been consolidated into a single estimate."

For determining the oral reference dose, EPA used a method other than the default factor for considering human variability. Instead, a 15- to 20-fold factor was used based on the uncertainty of the potency for mouse liver tumor production. These uncertainty factors for potency were assumed to be the same as the uncertainty in the internal dose estimates defined as the area under the curve for trichloroacetic acid and dichloroacetic acid, respectively. This 15- to 20-fold factor was assumed to account for both human pharmacokinetic variability and the pharmacokinetic differences between animals and humans.[b] The derivation of the 15- to 20-fold uncertainty factor was not thoroughly explained. EPA states: "A factor of 15-20 reflects the pharmacokinetic uncertainty in the liver between the 50th and 99th percentiles (see Table 9-1)." In the risk assessment, EPA supports this value with studies demonstrating that continuing exposure to trichloroethylene can increase the severity of effects. Somewhat paradoxically, the same studies were used to support the lesser $10^{1/2}$- fold uncertainty factor for the oral dose.

Table 9-1, reproduced below, is adapted from Table 15 of Rhomberg (2000), which details the uncertainty in human risks of liver tumors based on an analysis of mouse liver tumors using trichloroacetic acid and dichloroacetic acid as an internal dose measure. The uncertainty for human potency in producing liver tumors is estimated by assuming the toxic equivalency of internal dose (presented as the area under the curve) between humans and mice. The uncertainty

TABLE 9-1 Approximate Uncertainty Analysis Based on Log-Normal Error

	Uncertainty in potencies		
Human potency based on	Uncertainty in animal internal dose, GSD_A	Uncertainty in human internal dose, GSD_H	Uncertainty in human potency dose, GSD_{POT}
Mouse liver, TCA-auc	2.1	2.4	3.2
Mouse liver, DCA-auc	2.7	2.2	3.6
Rat kidney, thiol	3.4	6.2	9.0
Mouse lung, CH-auc	3	9	11.7
Mouse lung, CH-max	3.5	9	12.5

	Factor different from median estimate										
	Percentile of potency uncertainty distribution										
Human potency based on	1	2.5	5	10	25	50	75	90	95	97.5	99
Mouse liver, TCA-auc	1/15	1/10	1/7	1/4.4	1/2.2	1	2.2	4.4	7	10	15
Mouse liver, DCA-auc	1/20	1/12	1/8	1/5	1/2.4	1	5	5	8	12	20
Rat kidney, thiol	1/170	1/74	1/37	1/17	1/4.4	1	4.4	17	37	74	170
Mouse lung, CH-auc	1/300	1/120	1/56	1/23	1/5.2	1	5.2	23	56	120	300
Mouse lung, CH-max	1/360	140	1/63	1/25	1/5.4	1	5.4	25	63	140	360

Abbreviations: CH,chloral hydrate; DCA, dichloroacetic acid; TCA, trichloroacetic acid.
Source: Rhomberg 2000. Reprinted with permission; copyright 2000, Environmental Health Perspectives.

[b] From EPA (2001b, p. 4-7): Human variation: The NOAELs, LOAELs, and LED_{10}s for adverse liver effects were estimated using a pharmacokinetic model. The parameter uncertainty in these modeled dose estimates (estimated between the 50th and 99th percentiles, see Table 9-1) is 15-fold if plasma TCA [trichloroacetic acid] is used as the dose metric and 20-fold if plasma DCA [dichloroacetic acid] is used.

distributions for the animal internal dose and human internal dose—which were derived from a Bayesian uncertainty analysis (Bois 2000a) using the Clewell et al. (2000) model—were mathematically combined to estimate the distribution for the uncertainty in human potency. As mentioned, EPA used the fold difference between the 50th and 99th percentile to estimate both the variability in human pharmacokinetics and the uncertainty regarding the extrapolation of pharmacokinetic parameters from animals to humans. Problems with considering this as a measure of human pharmacokinetic variability include its derivation from mouse data, use of the Clewell model compared with others, and use of an assessment of the variability in cancer potency for assigning the variability of non-cancer effects.[c]

FINDINGS AND RECOMMENDATIONS

The scientifically appropriate inclusion of human variability into risk assessment is an ongoing challenge. EPA has attempted to account for human variability, particularly for vulnerable populations, with an array of uncertainty factors. EPA is encouraged to increase the precision of risk estimates used for fetuses and children with PBPK modeling approaches similar to that used for adults. Similar approaches also can be used to account for ethanol consumption and exposure to compounds with known metabolic interactions. Multiple suggestions are given below for additional data analysis and data generation, particularly to advance understanding of the role of genetic polymorphisms in trichloroethylene disposition as determinants of susceptibility. The committee questions whether the use of variability in animals to approximate human variability is appropriate.

Increased use of PBPK modeling in developmental risk assessment is essential for addressing health issues specific to children. This approach would increase the precision of risk assessment by enhancing the understanding of biologically relevant dosimetry related to fetal or pediatric exposures compared with adult exposures as well as, in some cases, allow for extrapolations across routes of exposure. In addition, available animal studies with blood concentrations could be used to model relevant target tissue concentrations (e.g., central nervous system or kidney) that are necessary to cause specific end point effects with identified modes of action. If such modeled target tissue concentrations were available for both animals and humans, it would enhance the ability to determine whether a developmental risk is plausible based on relevant tissue dosimetry. Within the developmental PBPK modeling, it is important to recognize that children do not represent a single group and that several physiologic stages must be considered.

It is unknown which human enzymatic isoforms dispose of trichloroethylene and its metabolites most efficiently. This information is critical for determining the relevance of various common functional genetic polymorphisms already known among enzyme families involved in trichloroethylene disposition as well as those that might be identified in the near future. Knowing the relevance of these genetic polymorphisms to risk assessment could then be

[c]While the risk assessment uses this uncertainty factor in the calculations for effects other than cancer, Rhomberg's analysis derives this uncertainty distribution for potency in producing liver tumors. In its analysis, EPA accepted the assumption that the uncertainty distribution of the internal dose at the lowest experimental exposure is a reasonable approximation of the uncertainty in low internal dose potency. However, the EPA assessment is not congruent with the fact that these uncertainty factors do not include estimates of human pharmacokinetic variation.

determined. Approaches include PBPK modeling such as that already performed for parathion and warfarin (Gentry et al. 2002).

PBPK models are needed, but do not address the well-recognized pharmacodynamic differences between children in adults. Intersubject variation in pharmacodynamic factors has not been well quantitated among adults and pharmacodynamic modeling of toxicant effects has not been performed. Further, modeling of long term end points of toxicant effects is difficult. For this to be attempted, the critical end points must be defined and appropriate shorter term effect biomarkers of these end points validated.

Recommendations: PBPK models for different physiologic stages of childhood development should be created for trichloroethylene. Research on children's exposure to trichloroethylene will be required to support model development, including measurement of trichloroethylene metabolites in breast milk and biological matrices from children (e.g., cord blood, amniotic fluid, and meconium) in different age groups.

- Improved information on dermal absorption and alterations in risk from developmental differences in skin thickness, as well as surface area and body weight determinations, is needed.
- If interspecies differences are determined to be predominantly related to compound disposition, PBPK models that incorporate critical comparative biology and physiology can be used to extrapolate developmental studies in animals to humans.
- More research is needed to understand which human enzymatic isoforms are most important in disposing trichloroethylene and its metabolites.
- Better characterization is needed of the impact of physiologic conditions and disease states on trichloroethylene toxicity, particularly with low-dose chronic exposure. It is possible that existing data sets could be mined for pertinent information, particularly for common disorders or factors, such as diabetes, obesity, and alcohol consumption.
- Additional data regarding intersubject variation in pharmacodynamic differences is needed across life stages and in various subpopulations before pharmacodynamic factors can be quantitated in risk assessment. Before such pharmacodynamic data can be generated, the critical targets and modes of action must be clarified from animal or in vitro studies.

10

Mixtures

Potential and known interactions between carcinogens and noncarcinogens in chemical mixtures in the environment have been a concern for several decades. Toxicokinetic and toxicodynamic interactions might result in decreased (antagonistic), exaggerated, additive, or unchanged toxicity relative to that of individual components. Although exaggerated toxicity is the primary concern, the antagonistic interaction resulting in decreased toxicity would also affect the assessment and management of risk.

Kidney and liver are the major target organs for trichloroethylene toxicity resulting from the generation of reactive metabolites through glutathione conjugation and cytochrome P-450-mediated metabolism. Human health risks of trichloroethylene stem mainly from its carcinogenic potential. In the body, trichloroethylene is metabolized into trichloroacetic acid, chloral hydrate, 2-chloroacetaldehyde, trichloroethanol, trichloroethanol glucuronide, and perhaps dichloroacetic acid. Because trichloroethylene is readily absorbed by all routes of exposure and extensively metabolized to multiple chemical species, exposure to trichloroethylene can be considered an exposure to a toxic mixture. Information on trichloroethylene metabolite toxicity is helpful in identifying the metabolites responsible for toxicity and might influence the effect of coexposures to other toxicants, particularly if they directly or indirectly change the proportions of trichloroethylene metabolites.

This chapter presents an overview of mixture toxicology, some of the important coexposure issues to consider in evaluating trichloroethylene, and possible approaches to using pharmacokinetic modeling for making predictions.

TOXICOLOGY OF MIXTURES CONTAINING TRICHLOROETHYLENE

Laboratory toxicity testing of single compounds can produce toxicity data specific to that compound for that species, but it cannot take into account the possible toxic effects of mixtures of compounds. For example, in a 6-month carcinogenicity assay, trichloroethylene-contaminated groundwater was found to be carcinogenic in Japanese medaka fish, after initiation with diethylnitrosamine (Gardner et al. 1998). Analysis of the groundwater indicated that contamination was not limited to trichloroethylene. No tumor promotional effect was found in a

follow-up laboratory study with reagent-grade trichloroethylene added to the groundwater to simulate the exposure concentration found in the contaminated groundwater. These studies implicate other water contaminants that might synergize the tumor-promoting activity of trichloroethylene.

Acute or repeated inhalation exposure to a mixture of 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene at concentrations as low as 20 parts per million (ppm) produced neurologic impairment. Male and female weanling ICR mice were treated with a mixture of chlorinated alkanes and alkenes consisting of chloroform, 1,1-dichloroethane, 1,1-dichloroethylene, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene in drinking water for 16 and 18 months, respectively; male mice developed hepatocelluar neoplasms and female mice developed mammary adenocarcinoma (Wang et al. 2002). The toxicokinetics of trichloroethylene was altered in rats receiving a binary mixture of chloroform and trichloroethylene (Anand et al. 2005a). Metabolism of trichloroethylene is suppressed in humans with coexposure to tetrachloroethylene (Seiji et al. 1989). Exposure to a ternary mixture of chloroform, trichloroethylene, and allyl alcohol results in less initial liver injury in male Sprague-Dawley rats because of greater elimination of trichloroethylene (Anand et al. 2005b).

A number of commonly used drugs modify the metabolism of trichloroethylene (Leibman and McAllister 1967; Carlson 1974; Moslen et al. 1977; Pessayre et al. 1979). The opposite might also occur, resulting in important modifications of the therapeutic action of the drugs (Kelley and Brown 1974). Trichloroethylene competitively inhibits the metabolism of barbiturates, producing exaggerated effects of the drugs (Kelley and Brown 1974). Sellers and Koch-Weser (1970) observed a potentiation of the anticoagulant effect of bishydroxycoumarin (warfarin) in patients after chloral hydrate ingestion, which appears to result from displacement of plasma protein binding sites by the chloral hydrate metabolite trichloroacetic acid. Trichloroacetic acid is extensively bound to plasma proteins (Templin et al. 1995), making it likely that trichloroethylene might potentiate the effects of many other drugs that normally bind to the same protein sites. Ethanol (2 g in daily liquid diet for 3 weeks) pretreatments enhanced hepatic damage in male Wistar rats treated with trichloroethylene (inhalation exposures of 500 ppm for 8 hours, 2,000 ppm for 2 or 8 hours, and 8,000 ppm for 2 hours) (Okino et al. 1991). Chemical coexposures from the environment in addition to human behaviors, such as alcohol consumption, might have effects that overlap with hepatic damage in male Wistar rats in terms of toxicokinetics, pharmacodynamics, and target tissue toxicity (see also Chapters 9 and 11). Alcohol consumption is a common coexposure that has been noted to affect trichloroethylene toxicity (see discussion later in this chapter). Coexposure to trichloroethylene might increase the toxicity of methanol and ethanol by altering their metabolism to aldehydes and also by altering their detoxification. Concomitant administration of alcohol and chloral hydrate in humans exacerbated the side effects of chloral hydrate (e.g., vasodilation, tachycardia, hypotension) (Sellers et al. 1972; Muller et al. 1975). The intolerance syndrome resulting from combined exposure to trichloroethylene and ethanol is due to increased accumulation of trichloroethylene in the central nervous system resulting from depression of trichloroethylene oxidation. Therefore, there is adequate basis for interactions to modulate the toxicity of trichloroethylene upon coexposure to other chemicals.

Interaction of metals with trichloroethylene could result in altered absorption of the metals. Dermal penetration of nickel significantly increased when it was administered along with phenol, toluene, and trichloroethylene to dermatomed male pig skin samples in flow-

through diffusion cells. Consequently, the potential health risk from dermal exposure to nickel is enhanced if other chemicals are present (Turkall et al. 2003). Not all metals interact with trichloroethylene in the same way. When lead carbonate and trichloroethylene were given concurrently to male rats, no additive or synergistic neurotoxicities were observed (Nunes et al. 2001).

Coexposures to trichloroethylene, trichloroacetic acid, and dichloroacetic acid at environmental concentrations are not uncommon (Wu and Schaum 2000). Trichloroethylene and tetrachloroethylene share common metabolites that have similar actions and targets and, therefore, coexposures potentially increase the risk from exposure to trichloroethylene. Trichloroethylene and di-(2-ethylhexyl)phthalate, a peroxisome proliferator, were reported to synergize prenatal loss, cause a decrease in pup weight, and cause anaophthalmia in rats (Narotsky and Kavlock 1995; Narotsky et al. 1995).

Veeramachaneni et al. (2001) reported effects in male rabbits exposed to drinking water containing chemicals at concentrations typical of groundwater near hazardous waste sites (the exposure mixture contained arsenic, chromium, lead, benzene, chloroform, phenol, and trichloroethylene). Even at 45 weeks after last exposure to drinking water pollutants, mating desire or ability, sperm quality, and Leydig cell function were subnormal. However, although the exposure concentrations are relevant to human environmental exposures, the design of this study precludes a conclusion about what combination of the seven toxicants, or what individual toxicant, caused the effects, exemplifying the problems associated with studying toxicology of a multicomponent mixture. Recent literature on interactions of trichloroethylene metabolites and common coexposures report the interactions of two or three chemicals at a time and use several approaches including examination of tumor phenotype, gene expression, and development of physiologically based pharmacokinetic (PBPK) models to assess possible synergy, antagonism, and additivity of effects or toxicokinetics. These studies may provide insights into possible modes of action and modulators of trichloroethylene toxicity.

One area that still hampers the risk assessment is interindividual differences that lead to variation in toxic responses in human populations. Although many factors are involved and the science still does not allow us to quantitate the influence of each factor, little is known about the influence of diet and caloric intake on trichloroethylene toxicity. A diet rich in carbohydrates protects male Wistar rats from liver injury by decelerating the transformation of trichloroethylene to highly toxic intermediates (Nakajima et al. 1982). A combination of ethanol with a low-carbohydrate diet accelerates the metabolism and enhancement of hepatotoxicity of trichloroethylene in male Wistar rats (Sato et al. 1983). A dietary copper imbalance resulted in higher trichloroethylene-induced lung damage as evidenced by a larger number of vacuolated Clara cells (Giovanetti et al. 1998).

Some of the important coexposures that affect the toxicity of trichloroethylene are discussed below.

Contaminants of Trichloroethylene

Earlier carcinogenic studies (NCI 1976) with trichloroethylene were faulted because they used commercial grade trichloroethylene as the test agent, which could contain stabilizers such as epichlorohydrin, a known carcinogen. Henschler et al. (1984) studied the effects of oral administration of trichloroethylene with and without stabilizers (epichlorohydrin and 1,2-

epoxybutane) on ICR/Ha Swiss mice. They concluded that there was an increase in forestomach cancers in the mice treated with trichloroethylene containing stabilizers, but there was no effect on the induction of liver tumors. They attributed the increase in forestomach cancers to the direct alkylating properties of epichlorohydrin and epoxybutane.

Interactions Between Trichloroacetic Acid and Dichloroacetic Acid

A recent study (Bull et al. 2002) attempted to examine how coexposures and variations in relative concentration between two trichloroethylene metabolites, dichloroacetic acid and trichloroacetic acid, might affect toxicity. Bull et al. (2002) reported that the tumor phenotype in $B6C3F_1$ male mice depended on the proportion of the two chemicals administered after 52 weeks of exposure. Given alone, trichloroacetic acid (0.5 or 2 g/L) and dichloroacetic acid (0.1, 0.5, or 2 g/L) produced liver tumors in mice with phenotypic characteristics that are distinct in several respects, with each compound at doses that were not cytotoxic. Combinations of trichloroacetic acid (0.5 or 2 g/L) and dichloroacetic acid (0.1 or 0.5 g/L) resulted in dose-related increases in hepatic preneoplastic lesions, adenomas, and carcinomas greater than either compound alone and in an additive fashion, with the addition of dichloroacetic acid to fixed exposures to trichloroacetic acid causing an increase in adenomas but not in carcinomas. Given alone, dichloroacetic acid produces tumors in mice that display a diffuse immunoreactivity to a c-*Jun* antibody, whereas trichloroacetic acid-induced tumors do not stain with this antibody. When given in various combinations, dichloroacetic acid and trichloroacetic acid produced a few c-Jun^+ tumors, and many that were c-Jun^-, but a number with a mixed phenotype increased with the dose of dichloroacetic acid. A comparison of tumor phenotypes induced by trichloroethylene (1 g/kg) shows that such tumors also have a mixture of phenotypes, suggesting that trichloroethylene-induced tumors are not consistent with either trichloroacetic acid or dichloroacetic acid acting alone.

Coexposures to Other Haloacetates

Other haloacetates produced in the bromination of drinking water might affect trichloroethylene toxicity through a similarity of effects of its metabolites. Kato-Weinstein et al. (2001) reported that brominated haloacetates such as bromodichloroacetate, bromochloroacetate, and dibromoacetate appear at higher concentrations in drinking water than the chlorinated haloacetates dichloroacetic acid and trichloroacetic acid. To study the similarity in action between the brominated and chlorinated haloacetates, mice were administered dibromoacetate, bromochloroacetate, and bromodichloroacetate in drinking water at concentrations of 0.2-3 g/L for 12 weeks (Tao et al. 2005). The dihaloacetates, bromochloroacetate and dibromoacetate, caused liver glycogen accumulation similar to that of dichloroacetic acid. The authors noted possible contamination of bromochloroacetate with dichloroacetic acid and dibromoacetate in their studies. The trihaloacetates, trichloroacetic acid and low concentrations of bromodichloroacetate, produced slight decreases in liver glycogen content, especially in the centrilobular region. The high concentration of bromodichloroacetate produced a pattern of glycogen distribution similar to that in dichloroacetic acid-treated mice. All dihaloacetates reduced the amount of serum insulin at high concentrations. Conversely, trihaloacetates had no

significant effects on serum insulin concentrations. After up to 26 weeks of treatment, dibromoacetate was the only brominated haloacetate that consistently increased acyl-coenzyme A oxidase activity (a marker of peroxisome proliferator-activated receptor alpha) agonism and rates of cell replication in the liver, but these effects were limited to 2-4 weeks of treatment and at exposures > 1 g/L (Tao et al. 2004a).

Coexposures to Other Solvents

Promotional and gene expression effects of trichloroethylene metabolites have been investigated in a number of studies in which they were administered after initial treatment with other carcinogens. Bull et al. (2004) studied interactions of metabolites (trichloroacetic acid and dichloroacetic acid) and carbon tetrachloride, motivated by the fact that trichloroethylene and carbon tetrachloride are commonly found together at contaminated sites. B6C3F$_1$ male mice, initially treated vinyl carbamate (3 mg/kg) at 2 weeks of age, were treated with dichloroacetic acid (0.1, 0.5, or 2.0 g/L), trichloroacetic acid (0.1, 0.5, or 2.0 g/L), and carbon tetrachloride (50, 100, and 500 mg/kg, and then reduced at week 24 to 5, 20, and 50 mg/kg due to toxicity) or pairwise combinations of the three compounds for 18-36 weeks. Histopathologically, a sample of 100 lesions was examined to verify that the criteria for the general descriptor of neoplastic and nonneoplastic lesions were satisfied. As the dose of carbon tetrachloride increased, the number of tumors per animal increased, whereas mean tumor size decreased. When administered alone in drinking water, dichloroacetic acid increased both tumor number and tumor size in a dose-related manner. With trichloroacetic acid treatment, tumor numbers plateaued by 24 weeks at a high dose. Dichloroacetic acid treatment did not produce a plateau in tumor number within the experimental period, but the numbers observed at the end of the experimental period (36 weeks) were similar to those found with trichloroacetic acid and to doses of carbon tetrachloride at 50 mg/kg.

Differing combinations of the three agents in initiated animals gave more complex results between 24 and 36 weeks of observation. At 24 weeks, dichloroacetic acid produced a decrease in tumor numbers promoted by trichloroacetic acid, but the numbers were not different from those for trichloroacetic acid alone at 36 weeks. The reason for this result became apparent at 36 weeks of treatment, when dichloroacetic acid coadministration led to a dose-related decrease in the size of tumors promoted by trichloroacetic acid. However, the low dose of trichloroacetic acid decreased the number of tumors produced by a high dose of dichloroacetic acid (2 g/L), but higher doses of trichloroacetic acid (2 g/L) produced the same number that was observed with dichloroacetic acid alone. Dichloroacetic acid inhibited the growth rate of carbon tetrachloride-induced tumors. Trichloroacetic acid substantially increased the number of tumors observed at early time points when combined with carbon tetrachloride, but this effect was not observed at 36 weeks. The lack of an effect at 36 weeks was attributed to the fact that more than 90% of the livers consisted of tumors and the earlier effect was masked by coalescence of the tumors. Thus, trichloroacetic acid significantly increased tumor numbers in mice treated with carbon tetrachloride.

Pretreatment with trichloroethylene in drinking water at concentrations as low as 15 mM for 3 days has also been reported to increase susceptibility to liver damage to subsequent exposure to a single intraperitoneal injection (1 mL/kg) of carbon tetrachloride in Fischer 344 rats (Steup et al. 1991). Several mechanistic hypotheses offered included altered metabolism,

decreased hepatic repair capability, decreased detoxification ability, or a combination of these. Simultaneous administration of trichloroethylene (0.5 mL/kg) also increased the liver injury induced by carbon tetrachloride (0.05 mL/kg) (Steup et al. 1993). The authors suggested that trichloroethylene appeared to impair the regenerative activity in the liver, thus leading to increased damage when carbon tetrachloride is given in combination with trichloroethylene.

Chloroform, a chlorine disinfection by-product found in drinking water, as well as dichloroacetic acid and trichloroacetic acid, is also a mouse liver carcinogen and was the focus of another study by Pereira et al. (2001). They reported the effects of coexposure to chloroform (0, 400, 800, 1,600 mg/L) on hypomethylation and expression of the c-*myc* gene induced by treatment with dichloroacetic acid and trichloroacetic acid (500 mg/kg) in the livers of female $B6C3F_1$ mice. Dichloroacetic acid, trichloroacetic acid, and to a lesser extent chloroform decreased methylation of the c-*myc* gene. Coadministering chloroform (at 800 and 1,600 mg/L) decreased dichloroacetic acid-induced hypomethylation but it had no effect on that of trichloroacetic acid. Expression of c-*myc* mRNA was increased by dichloroacetic acid and trichloroacetic acid, with the two highest doses of chloroform attenuating the actions of dichloroacetic acid but not trichloroacetic acid.

In the same study, male and female $B6C3F_1$ mice, administered *N*-methyl-*N*-nitrosourea (an initiator of liver and kidney tumors) on day 15 of age, and dichloroacetic acid (3.2 g/L) or trichloroacetic acid (4.0 g/L) with chloroform (0, 800, or 1,600 mg/L) starting at 5 weeks of age, were examined after 36 weeks for promotion of liver and kidney tumors (Pereira et al. 2001). However, the numbers of animals in the group treated with *N*-methyl-*N*-nitrosourea, dichloroacetic acid, and chloroform and in the group treated with *N*-methyl-*N*-nitrosourea, trichloroacetic acid, and chloroform were variable and small (n = 6-8 in the female group), limiting the power of the study. In female mice, coexposure to 800 and 1,600 mg/L decreased the number of adenomas induced by *N*-methyl-*N*-nitrosourea and dichloroacetic acid, with no effect on carcinomas or on adenomas and carcinomas in the liver induced by *N*-methyl-*N*-nitrosourea and trichloroacetic acid. *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment resulted in no carcinoma induction in females. Only one animal had carcinomas induced by *N*-methyl-*N*-nitrosourea, dichloroacetic acid, and chloroform treatment. In male mice, *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment induced carcinomas as well as adenomas in the liver, with chloroform coexposure (at high concentrations) having no effect on the numbers of animals with adenomas and a reduction in those with carcinomas. Only the highest concentration of chloroform appeared to decrease the number of animals with adenomas in the trichloroacetic acid-treated group. No foci of altered hepatocytes were found in *N*-methyl-*N*-nitrosourea-initiated control mice of either sex. A larger number of foci of altered hepatocytes were seen in female than in male mice after *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment, although the number of tumors per mouse was about the same. Chloroform decreased the number of foci of altered hepatocytes and tumors per mouse at the two highest doses of dichloroacetic acid treatment in females and at the highest doses in males. Trichloroacetic acid induced few foci in female or male mice, with chloroform having no effect on foci of altered hepatocyte formation or tumor induction. Liver tumors and foci of altered hepatocytes were characterized as basophilic or eosinophilic. In females, both foci of altered hepatocytes and tumors were eosinophilic after *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment, whereas in males only foci of altered hepatocytes were eosinophilic, with tumors being basophilic. Coexposure to chloroform increased the percentage of foci of altered hepatocytes in males that were basophilic. Liver foci of altered hepatocytes and tumors induced by *N*-methyl-

N-nitrosourea and trichloroacetic acid treatment were basophilic in both sexes, with methyl chloroform having no effect. These results are consistent with those of Latendresse and Pereira (1997), who reported that, after initiation of *N*-methyl-*N*-nitrosourea, dichloroacetic acid-induced foci of altered hepatocytes and tumors in female mice were eosinophilic and stained positively for transforming growth factor alpha, c-*jun*, c-*myc*, CYP2E1, CYP4A1, and glutathione *S*-transferase (GST)-pi, while trichloroacetic acid treatment induced foci of altered hepatocytes and tumors that were predominantly basophilic, lacked GST-pi, and stained variably for other biomarkers.

Pereira et al. (2001) also reported promotion of kidney tumors in male mice from dichloroacetic acid, trichloroacetic acid, and chloroform coexposures. The pattern of tumors in the kidneys were different than in the liver. No kidney tumors were initiated in male mice after treatment with *N*-methyl-*N*-nitrosourea alone or with chloroform coexposure. However, trichloroacetic acid increased the incidence (90%) and multiplicity of kidney tumors initiated by *N*-methyl-*N*-nitrosourea. Coexposure of chloroform with trichloroacetic acid had no effect on tumor incidence or multiplicity. Dichloroacetic acid alone did not significantly increase the incidence (24%) or multiplicity of *N*-methyl-*N*-nitrosourea-initiated kidney tumors, but coexposure with chloroform increased the incidence of kidney tumors to 100% in male mice. In female mice, kidney tumor incidence and multiplicity after trichloroacetic acid or dichloroacetic acid treatment with or without chloroform was low after initiation with *N*-methyl-*N*-nitrosourea.

Using a single-dose exposure, the toxicity of a quaternary mixture of trichloroethylene, allyl alcohol, chloroform, and thioacetamide, structurally dissimilar toxicants with dissimilar mechanisms by which they initiate liver injury, was tested and compared with the toxicity of individual components and the sum of their toxic effects in male Wistar rats (Soni et al. 1999). Also, the liver reparative responses to injury initiated by each component, and the sum of their effects, were compared with the response after exposure to the quaternary mixture. The combined toxic effects were additive, primarily because of a dose-related stimulation of liver reparative response that prevented progression and expansion of liver injury. The studies showed that the extent of injury at early time points correlates well with maximal stimulation of the liver tissue repair response suggesting that, in addition to initiation of tissue injury, the toxicodynamics of cell birth and tissue repair should be considered in evaluating the final toxic outcome.

Trichloroethylene and Tetrachloroethylene

Trichloroethylene and tetrachloroethylene are often found together as environmental contaminants, are metabolized by the same enzymes, and have similar metabolites (Green 1990). There are significant differences in the kinetics of metabolism of trichloroethylene and tetrachloroethylene by certain enzymes and in the chemical reactivity of certain analogous metabolites (IARC 1995a). Tetrachloroethylene metabolites are also formed in oxidative metabolism of trichloroethylene. But trichloroethanol and chloral are less important metabolites in tetrachloroethylene than in trichloroethylene metabolism. Tetrachloroethylene appears to be a much poorer substrate for cytochrome P-450 than its congener trichloroethylene (Ohtsuki et al. 1983; Volkel and Dekant 1998; Volkel et al. 1998). Hence, the various cytochrome P-450-derived metabolites from tetrachloroethylene and trichloroethylene will be produced at different rates. In vivo, tetrachloroethylene is conjugated with reduced glutathione (GSH) more

extensively (1% to 2% of the dose) (Dekant et al. 1986a) than trichloroethylene (<0.05% of the dose) (Green et al. 1997a). In humans, the GSH conjugation pathway is toxicologically significant only at high doses or when the cytochrome P-450 pathway is saturated with trichloroethylene and tetrachloroethylene (Green 1990; Green et al. 1990). The glutathione pathway plays a greater role in tetrachloroethylene metabolism than in trichloroethylene metabolism. Chloral hydrate, a metabolite of both tetrachloroethylene and trichloroethylene, produces liver tumors in B6C3F$_1$ mice (Rijhsinghani et al. 1986). Although chloral hydrate is the predominant intermediate in cytochrome P-450 metabolism of trichloroethylene, it is a minor intermediate in tetrachloroethylene metabolism (Lash and Parker 2001). Such differences in rates of metabolism of trichloroethylene and tetrachloroethylene and in mode of action imply that their risk hazards differ even though the same metabolites occur with both compounds. A nongenotoxic mode of action plays an important role in liver tumorogenesis induced by trichloroethylene and tetrachloroethylene in B6C3F$_1$ mice. Tetrachloroethylene showed a higher degree of cytotoxicity than trichloroethylene in kidney cells isolated from male rats (Lash and Parker 2001). Low doses of trichloroethylene (5-20 μL) or tetrachloroethylene (1-5 μL) significantly enhanced the intracellular GSH concentration. However, the concentration of GSH rapidly decreased with higher doses of trichloroethylene (40-80 μL) or tetrachloroethylene (10-20 μL) (Wang et al. 2001).

Trichloroethylene and Ethanol

Because trichloroethylene and ethanol have common metabolic pathways and the liver is the main site of metabolism for both compounds, there is special interest in understanding whether individuals exposed to trichloroethylene who also consume alcohol regularly are at greater risk for developing target organ toxicity and cancer. Lower tolerance to the inebriating effects of alcohol among workers exposed to trichloroethylene has been well-documented. A condition known as “degreasers flush” is seen in subjects exposed to trichloroethylene, where dilation of blood vessels in the skin surface occurs with consumption of small amounts of alcohol (Stewart et al. 1974).

The interaction between alcohol and trichloroethylene is very complex. The outcome of this interaction depends on whether there is simultaneous or alternate exposure to the two compounds. This interaction could involve: (1) direct competition between ethanol, trichloroethylene, and its metabolites for drug-metabolizing enzymes; (2) increased expression and activity of liver CYP2E1 by alcohol consumption, which is known to affect trichloroethylene metabolism; (3) changes in availability of cofactors for enzymes catalyzing the reductive and oxidative metabolism of trichloroethylene that occurs as a result of oxidative metabolism of ethanol; and (4) abnormal generation of reactive oxygen species by induced CYP2E1, which could synergize the adverse effects of trichloroethylene metabolites.

Trichloroethylene undergoes oxidation to chloral hydrate by the action of CYP2E1. Chloral hydrate then undergoes conversion to trichloroacetic acid. This is an oxidative reaction catalyzed by aldehyde dehydrogenase, which requires oxidized nicotinamide adenine dinucleotide (NAD) as cofactor. Alternatively, chloral hydrate can be converted to trichloroethanol by alcohol dehydrogenase. This reductive reaction requires reduced nicotinamide adenine dinucleotide (NADH). Ethanol uses the same two pathways for its consecutive oxidation to acetaldehyde and acetic acid, respectively.

Oxidation by CYP450 Versus GSH Conjugation Via GST: Changes in the Contribution of These Pathways to Trichloroethylene Metabolism by Ethanol

Microsomal metabolism of ethanol via CYP2E1 occurs more prominently relative to alcohol dehydrogenase with chronic alcohol use (Lieber 2004). Competition for CYP2E1 during coexposure to ethanol and trichloroethylene can reduce the conversion of trichloroethylene to chloral hydrate (Muller et al. 1975). By blocking CYP2E1, less conversion of trichloroethylene to chloral hydrate can increase the narcotic and solvent effects of trichloroethylene in various tissues. This interference with CYP2E1 can also shift the metabolism of trichloroethylene into the glutathione pathway (Sato and Nakajima 1985), resulting in generation of more glutathione-derived adducts of trichloroethylene. Generation of more of these conjugates can alter the susceptibility of exposed subjects to the adverse effects of trichloroethylene in kidneys because greater generation and delivery of *S*-1,2-dichlorovinyl-L-cysteine to this organ can be detrimental. This metabolite has been linked to both acute tubular necrosis (Gandolfi et al. 1981; Vaidya et al. 2003a) and cancer formation by trichloroethylene. These findings are based primarily on animal studies. Additional experimentation is needed to determine whether this shift in trichloroethylene metabolism occurs with ethanol coexposure and what its toxicologic significance is in humans.

Glutathione-mediated metabolism of trichloroethylene in humans is considered a minor pathway compared wtih its oxidative metabolism. This is in contrast to rodents, which are considered more susceptible to the acute nephrotoxicity and nephrocarcinogenicity of trichloroethylene. Accordingly, conjugation of trichloroethylene with glutathione is more prominent in rodents than in humans (Green et al. 1997a; Lash et al. 2000a). Bernauer et al. (1996) analyzed the urine of rats and humans for the presence of the *N*-acetylated metabolite of *S*-1,2-dichlorovinyl-L-cysteine after trichloroethylene exposure via inhalation. Urinary excretion of this metabolite was compared with that of products of the oxidative metabolites of trichloroethylene. The results showed that the urinary content of *N*-acetylated *S*-1,2-dichlorovinyl-L-cysteine in humans was 1,000-7,000 times lower than that for trichloroethanol and trichloroacetic acid (Bernauer et al. 1996).

The existing data suggest that GSH conjugation is a minor pathway for the metabolism of trichloroethylene in humans, but there is no indication of whether this pathway becomes more prominent during coexposure to ethanol and trichloroethylene, when ethanol metabolism impairs the oxidative metabolism of trichloroethylene via CYP2E1. By contrast, exposure to trichloroethylene alone in alcohol users is expected to have contrasting effects on the ability of the liver to metabolize trichloroethylene. With CYP2E1 induction, chloral hydrate formation during abstinence from alcohol consumption is expected to be higher, which should lead to enhanced generation of oxidative and conjugative trichloroethylene metabolites. Several animal studies have shown that to be the case. On the other hand, human studies documenting this finding are scarce.

Shift in Reducing Equivalents During Alcohol Metabolism

Ethanol metabolism is also known to shift the balance of reducing equivalents in hepatocytes (Kalant et al. 1970). A shift in the ratio of NAD^+ to NADH in favor of the reduced pyridine nucleotide takes place during alcohol metabolism. This higher reducing environment in

hepatocytes is known to affect the oxidative metabolism of trichloroethylene, as illustrated in studies by Larson and Bull (1989) where coadministration of ethanol and trichloroethylene to male Sprague-Dawley rats resulted in decreased blood concentrations of trichloroacetic acid compared with animals receiving trichloroethylene alone. Generation of trichloroacetic acid depends on NAD^+ availability, which is lower during alcohol metabolism. Ethanol coexposure also increases the urinary excretion ratio for trichloroethanol/trichloroacetic acid (Larson and Bull 1989). The authors of the study pointed out that this effect was pronounced only when very high doses of trichloroethylene and ethanol were used. Nevertheless, the study shows that a larger supply of reducing equivalents by alcohol metabolism favors the formation of trichloroethanol over trichloroacetic acid, which was highly predictable based on the form of NADH needed to catalyze the different chloral hydrate biotransformation reactions. The effect of ethanol on trichloroethylene metabolism has also been investigated in isolated perfused rat livers (Watanabe et al. 1998). The results of these liver perfusion studies are similar to those reported by Larson and Bull (1989).

The change in the NAD^+-to-NADH ratio produced by ethanol oxidation has another implication for exposure to mixtures beyond the changes in activity of metabolic pathways for trichloroethylene just described. This shift in reducing equivalents resulting from NADH accumulation also increases mitochondrial superoxide production by accelerating the flow of electrons down the respiratory electron transport chain (Koch et al. 2004). This, along with a higher production of reactive oxygen species under conditions of CYP2E1 induction, can enhance the susceptibility of the liver and other target organs to lipid peroxidation and oxidative damage to DNA produced by trichloroethylene and its metabolites. This shift in reducing equivalents produced by alcohol metabolism and NADH accumulation has also been implicated in some pathologic findings of alcoholic liver disease, including inhibition of fatty acid oxidation and steatosis.

CYP2E1 Induction and Oxidative Stress

The effect of alcohol use on microsomal metabolism and CYP2E1 expression deserves more in depth attention. The biochemical and toxicologic features of CYP2E1, as they relate to alcohol metabolism and toxicity, were recently reviewed by Caro and Cederbaum (2004). A decade ago, Cederbaum's group developed a human hepatoma HepG2 cell line with constitutive expression of CYP2E1. The parental cell line lacks any detectable CYP2E1. Overexpression of CYP2E1 in HepG2 cells results in a 50% increase in production of reactive oxygen species compared with untransfected cells. Associated with this, CYP2E1-expressing cells also exhibited increased lipid peroxidation and a significant decrease in cell proliferation that is possibly due to mitochondrial damage inflicted by CYP2E1-induced oxidative stress. It is worth noting that the enhanced oxidative stress in transfected cells occurs in the absence of added toxicant, which indicates that CYP2E1 expression by itself is responsible for this effect.

Although ethanol oxidation by liver alcohol dehydrogenase is the rate-limiting step in the total oxidation of this alcohol, ethanol oxidation also occurs via CYP450. This alternative metabolic pathway for ethanol is more pronounced with chronic alcohol consumption due to the well-documented CYP2E1 induction that occurs with chronic exposure. Multiple reviews have described this phenomenon and the mechanism involved in CYP2E1 induction (Lieber 2004). Normal CYP2E1 enzymatic activity generates reactive oxygen species such as superoxide and

hydrogen peroxide in higher amounts than other CYP450 isoforms (Gorsky et al. 1984). With ethanol induction of hepatic CYP2E1, the enhanced formation of reactive oxygen species resulting from normal CYP2E1 catalysis has been linked to development of chronic alcoholic liver disease. Most importantly, in vivo and in vitro studies with freshly isolated hepatocytes have also demonstrated that ethanol exposure can produce oxidative stress and hepatocellular injury.

CYP2E1 induction by ethanol has dual implications to toxicity resulting from exposure to mixtures consisting of ethanol and other xenobiotics. Enhanced expression of CYP2E1 influences not only the toxicologic potency of xenobiotics by altering the profile of metabolites that are generated but also the formation of reactive oxygen species that occurs with CYP2E1 induction can potentiate the toxic effects of xenobiotics that work by generating reactive oxygen species. These considerations are highly relevant to trichloroethylene because alcohol consumption has been reported to affect trichloroethylene metabolism and also its hepatotoxicity (Nakajima et al. 1988; Okino et al. 1991). In summary, two sources of potentially damaging reactive oxygen species have been presented in relation to alcohol consumption: (1) one coming from NADH accumulation, which stimulates mitochondrial superoxide generation, and (2) a second one originating from induced CYP2E1 enzymatic activity. Reactive nitrogen species is another category of damaging intermediates produced in response to alcohol consumption (see below).

Ethanol and Nitric Oxide Production: Changes in Blood Flow and Peroxynitrite Formation

The enhanced production of nitric oxide that occurs in association with alcohol consumption can also affect the toxicity of trichloroethylene and its metabolites. Ethanol increases blood flow to selected organs, such as the kidney and liver, without affecting perfusion to other tissues like the brain and lungs. This effect appears to be mediated by a stimulation of nitric oxide production (Baraona et al. 2002a). However, there are conflicting reports on the effect of ethanol on the activity of inducible nitric oxide synthase. Some investigations indicate that ethanol induces nitric oxide synthase activity (Baraona et al. 2002a,b), but a recent study in rats showed that consuming a liquid diet containing 3% ethanol (vol/vol) for 12 weeks reduced hepatic inducible nitric oxide synthase activity significantly (Wang and Abdel-Rahman 2005). These results are inconsistent with higher nitric oxide generation. Regardless of the mechanism involved, increased production of nitric oxide by ethanol has dual implications for the toxicity of other xenobiotics. Changes in blood perfusion rates to selected organs can lead to changes in pharmacokinetic and pharmacodynamic parameters for xenobiotics in alcohol users. Second, increased production of nitric oxide leads to secondary production of peroxynitrite. This reactive nitrogen intermediate has been shown to cause protein nitration and tissue injury (Jaeschke et al. 2002). The combined effect of peroxynitrite and reactive oxygen species generated in response to alcohol consumption can synergize the cytotoxic potential of trichloroethylene.

Implications

Ethanol coexposure can change the biotransformation and disposition of trichloroethylene through three distinct mechanisms: (1) by direct competition between chloral

hydrate and ethanol or its oxidative product acetaldehyde for alcohol or aldehyde dehydrogenase, (2) by changing the ratio of pyridine dinucleotide cofactors needed to convert chloral hydrate to trichloroacetic acid or trichloroethanol, and (3) by direct competition between trichloroethylene and ethanol for the active site of CYP2E1.

Greater availability of NADH favors the conversion of chloral hydrate to trichloroethanol, which is considered to be a noncarcinogenic metabolite of trichloroethylene. The significance to human health of this shift in metabolism is not known because most reports documenting this effect were generated with rodents. Simultaneous metabolism of trichloroethylene and ethanol by CYP2E1 can shift more of the trichloroethylene metabolism into the GST-conjugation pathway. In turn, higher generation of GSH-derived adducts of trichloroethylene (including *S*-1,2-dichlorovinyl-L-cysteine) can alter the susceptibility of the kidneys to acute necrosis and cancer. Once again, the significance of this interaction in human health is unknown.

On the other hand, exposure to trichloroethylene after alcohol consumption in habitual drinkers represents another chemical interaction with mechanistic features that are distinct from the coexposure situation. As a result of CYP2E1 induction in alcohol users, trichloroethylene metabolism to chloral hydrate proceeds faster when ethanol is not present. This has been documented in rat studies in which pretreatment with ethanol resulted in increased urinary excretion of CYP450-derived metabolites of trichloroethylene, trichloroacetic acid, and trichloroethanol (Nakajima et al. 1988). This was associated with more pronounced hepatotoxicity.

There is a large volume of data documenting this interaction between ethanol and trichloroethylene, where both metabolism and pattern of toxicity by trichloroethylene are changed. However, the bulk of this information is limited to studies using laboratory animals. Although some of the human data suggest that this interaction can occur in the workplace, its significance to alterations in patterns of trichloroethylene toxicity and cancer is unknown and deserves further attention.

POTENTIAL MECHANISMS OF INTERACTION

Bartonicek and Teisinger (1962) showed that disulfiram markedly inhibits the terminal enzymatic steps (detoxification) of trichloroethylene metabolism, resulting in enhanced trichloroethylene toxicity. Trichloroethylene induces CYP2E1 and inhibits alcohol dehydrogenase (Wang et al. 1999). Chloroform, when coadministered with dichloroacetic acid and trichloroacetic acid (metabolites of trichloroethylene), promoted kidney tumors in male mice by preventing hypomethylation of DNA and increasing mRNA expression of the c-*myc* gene (Pereira et al. 2001). The inductive and inhibitory effects of trichloroethylene on CYP2E1 and alcohol dehydrogenase, respectively, might result in different effects on the metabolism of other chemicals when coadministered with trichloroethylene. Besides a toxic response, tissue repair, a simultaneous biological compensatory response that accompanies chemical-induced injury, also plays an important role in mixture toxicity (Anand et al. 2005a,b). Trichloroethylene potentiates the hepatotoxicity of carbon tetrachloride by increasing carbon tetrachloride-induced lipid peroxidation (Pessayre et al. 1982).

Recent studies (Vaidya et al. 2003b,c; Korrapati et al. 2005) suggest another potential mechanism of altered toxicity upon coexposure to other toxicants. Renal tissue repair was

inhibited by a high dose of *S*-(1,2-dichlorovinyl)-L-cysteine (75 mg/kg, intraperitoneally) due to down-regulation of the IL-6/STAT-3 or the IL-6/ERK1/2 pathways causing cell cycle arrest at the beginning of the G_1- to S-phase transition (Vaidya et al. 2003c). Downstream of the ERK1/2 pathway, a high dose of *S*-(1,2-dichlorovinyl)-L-cysteine inhibits phosphorylation of IkBα, resulting in limited nuclear translocation of NF-kB. A cdk4/cdk6 system-mediated phosphorylation of retinoblastoma protein was down-regulated due to overexpression of p16 (Korrapati et al. 2005). Prior administration of a low priming dose of *S*-(1,2-dichlorovinyl)-L-cysteine (15 mg/kg) protects mice from a later lethal dose of *S*-(1,2-dichlorovinyl)-L-cysteine (75 mg/kg) (Vaidya et al. 2003b). A low dose of *S*-(1,2-dichlorovinyl)-L-cysteine exhibits prompt renal tubular regeneration by timely and adequate stimulation of IL-6, TGF-α, HB-EGF, EGFr, IGF-1R*β*, and phosphorylated ERK1/2, leading to recovery from a lethal dose challenge (Vaidya et al. 2003c). A priming dose led to higher expression of cyclin D1/cdk4-cdk6 downsteam, resulting in increased phosphorylation of retinoblastoma protein (Korrapati et al. 2005). Coexposure to other toxicants may result in interactions with the cellular signaling mechanisms affecting the response to trichloroethylene and, conversely, trichloroethylene (or its metabolites) might interfere with the cellular signaling mechanisms and cellular responses. Effects of chronic exposure to trichloroethylene or its metabolites on cellular signaling mechanisms and how they might be altered upon coexposure to other toxicants are not known.

EFFECTS OF ALTERED OR SPECIAL PHYSIOLOGIC STATES

Studies of exposure to trichloroethylene suggest a concern about reproductive issues and congenital heart defects (see Chapter 5 for complete discussion). For mixtures containing trichloroethylene, an increase in miscarriages has been reported among nurses exposed to unspecified concentrations of trichloroethylene and other chemicals in operating rooms (Corbett et al. 1974). Early exposure of male rabbits to a mixture of arsenic, chromium, lead, benzene, chloroform, phenol, and trichloroethylene in drinking water caused acrosomal dysgenesis, nuclear malformations, lower testosterone secretion, subnormal mating desire and ability, lower sperm quality, and decreased Leydig cell function (Veeramachaneni et al. 2001). Simultaneous oral administration of trichloroethylene (0.5 mL/kg) resulted in a marked potentiation of liver injury caused by an oral dose of chloroform (0.05 mL/kg) due to delayed hepatic regeneration (Steup et al. 1993). Pretreatment with drinking water solutions containing trichloroethylene or chloroform enhances the hepatotoxicity of carbon tetrachloride in Fischer 344 rats (Steup et al. 1991). Inhalation of small concentrations of petroleum and trichloroethylene caused degenerative changes in the hepatic parenchyma cells in pregnant female Wistar rats (Duricic and Duricic 1991).

COEXPOSURE PREDICTIONS USING PBPK MODELS

An important issue is whether and the degree to which modulation of toxicity by coexposures can be quantified. PBPK models have been developed to predict possible synergy, antagonism, and additivity of effects on pharmacokinetics. Given that trichloroethylene, tetrachloroethylene, and methyl chloroform are often found together in contaminated groundwater, Dobrev et al. (2001) attempted to investigate the pharmacokinetic interactions

among the three solvents to calculate defined "interaction thresholds" for effects on metabolism and expected toxicity. Their null hypothesis was defined as competitive metabolic inhibition being the predominant result for trichloroethylene given in combination with other solvents. They used gas uptake inhalation studies to test different inhibition mechanisms. A PBPK model was developed with the gas uptake data to test multiple mechanisms of inhibitory interactions (competitive, noncompetitive, or uncompetitive) with the authors reporting competitive inhibition of trichloroethylene metabolism by methyl chloroform and tetrachloroethylene in simulations of pharmacokinetics in rats. Occupational exposures to chemical mixtures of the three solvents within their threshold limit value or time-weighted average limits were predicted to result in a significant increase (22%) in trichloroethylene blood concentrations compared with single exposures.

Dobrev et al. (2002) extended this work to humans by developing an interactive human PBPK model to explore the general pharmacokinetic profile of two common biomarkers of exposure: peak trichloroethylene blood concentrations and total trichloroethylene metabolites generated in rats and humans. Increases in the trichloroethylene blood concentrations were predicted to lead to greater availability of the parent compound for glutathione conjugation, a metabolic pathway that may be associated with kidney toxicity or carcinogenicity. A fractional change in trichloroethylene blood concentration of 15% for a combined threshold limit value for exposure to the three chemicals (25, 50, and 350 ppm of tetrachloroethylene, trichloroethylene, and methyl chloroform, respectively) resulted in a 27% increase in *S*-(1, 2-dichlorovinyl)-L-cysteine metabolites, indicating a nonlinear risk increase due to combined exposures to trichloroethylene. Binary combinations of the solvents produced glutathione-mediated metabolite amounts almost twice as high as the expected rates of increase in the parent compound blood concentrations. The authors suggested that using parent blood concentrations (a less sensitive biomarker) would result in two to three times higher (less conservative) estimates of potentially safe exposures. For detecting metabolic inhibition from tetrachloroethylene and methyl chloroform, the simulations showed trichloroethylene blood concentrations to be the more sensitive dose metric in rats, but the total of trichloroethylene metabolites was a more sensitive dose measure in humans. Finally, interaction thresholds were predicted to occur at lower concentrations in humans than in rats.

Thrall and Poet (2000) investigated the pharmacokinetic impact of low-dose coexposures to toluene and trichloroethylene in male F344 rats in vivo using a real-time breath analysis system coupled with PBPK modeling. The authors reported that, using the binary mixture to compare the measured exhaled breath concentrations from high- and low-dose exposures with the predicted concentrations under various metabolic interaction simulations (competitive, noncompetitive, or uncompetitive inhibition), the optimized competitive metabolic interaction description yielded an interaction parameter K_i value closest to the Michaelis-Menten affinity parameter (K_m) of the inhibitor solvent. They suggested that competitive inhibition is the most plausible type of metabolic interaction between these two solvents.

Isaacs et al. (2004) reported gas uptake coexposure data for chloroform and trichloroethylene. They questioned whether it was possible to use inhalation data in combination with PBPK modeling to distinguish between different metabolic interactions using sensitivity analysis theory. They reported that chloroform and trichloroethylene act as competitive inhibitors of each other's metabolism. Recommendations were made for the design of efficient experiments aimed at determining the type of inhibition mechanisms resulting from a binary coexposure protocol. Even though, as stated by Dobrev et al. (2002), other solvents inhibit

trichloroethylene metabolism, it is possible to quantify the synergistic interaction of trichloroethylene on other solvents with techniques such as gas uptake inhalation exposures.

Haddad et al. (2000) developed a theoretical approach to predict the maximum impact that a mixture consisting of coexposure to dichloromethane; benzene; trichloroethylene; toluene; tetrachloroethylene; ethylbenzene; *m*-, *p*-, and *o*-xylene; and styrene would have on venous blood concentration due to metabolic interactions in Sprague-Dawley rats. They conducted two sets of experimental coexposures. The first study evaluated the change in venous blood concentration after a 4-hour constant inhalation exposure to the 10-chemical mixture. The second study was designed to examine the impact of possible enzyme induction by using the same inhalation coexposure after a 3-day pretreatment with the same 10-chemical mixture. The resulting venous concentration measurements for trichloroethylene from the first study were consistent with metabolic inhibition. The 10-chemical mixture was the most complex coexposure used in this study. The authors stated that resulting parent concentration time courses change less as mixture complexity increases, an observation consistent with metabolic inhibition. For the pretreatment study, the authors found a systematic decrease in venous concentration (due to higher metabolic clearance) for all chemicals except tetrachloroethylene. Overall, these studies suggest a complex metabolic interaction between trichloroethylene and other solvents.

A PBPK model for trichloroethylene including all its metabolites and their interactions can be considered a mixture model in which all metabolites have a common starting point in the liver. An integrated approach is needed after taking into account trichloroethylene metabolites and their interactions with each other, including inhibition of metabolites.

FINDINGS AND RECOMMENDATIONS

Although the available data indicate that toxic effects of trichloroethylene and its metabolites are likely to change in the presence of exposure to other chemicals, including its metabolites and similar metabolites of other toxicants, a definitive understanding of whether and which of the toxic effects might be increased, decreased, or unchanged is lacking. Much of this must come from research in animals or other biosystems, because in humans, exposures to other compounds and factors would be difficult to obtain accurately and reliably in humans. The present state of knowledge does allow identifying the major potential mechanisms as the basis of such interactions at the biophase, but to what extent and how they could influence the toxicity outcomes cannot be predicted. Examples of such mechanisms are altered xenobiotic metabolizing enzymes, toxicokinetic factors (absorption, distribution, and elimination), toxic metabolite accumulation in target and nontarget tissues, and toxicodynamic factors, such as cell death, proliferation, expression of survival factors, and epigenetic and genotoxic mechanisms.

Recommendations: Toxicokinetic and toxicodynamic studies are needed with mixtures to evaluate the effect of coexposures to other chemicals on toxic outcomes of trichloroethylene and on the toxicity of other coexposed toxicants including metabolites of trichloroethylene.

- Important toxic outcomes of trichloroethylene might be selected as end points for these studies. Species differences should be investigated.
- Testing large numbers and doses of compounds is not practical. Studies designed to learn more about mechanisms and modes of action in the presence of the most commonly occurring toxicants are likely to yield the most meaningful results.

- Testing to evaluate the impact of lifestyle factors, such as alcohol consumption, smoking, chronic drug intake, and diet (e.g., nutrition, caloric restriction) should be performed.
- Testing of mixtures to evaluate the impact of disease (e.g., diabetes) and special physiologic states (e.g., pregnancy, aging) should be performed.

11

Pharmacokinetic Modeling

Pharmacokinetic models describe the absorption, distribution, metabolism, and elimination of a chemical in an organism. Depending on the complexity of a pharmacokinetic model and the available data upon which it is based, the model can be used to predict the concentration of a parent chemical and metabolite(s) in various tissues, organs, cells, and subcellular compartments given any particular exposure pattern over time. Because target organ doses are more relevant to toxicity than the amount of exposure at a particular exterior boundary, pharmacokinetic models may be useful for assessing human health risk from exposure to a chemical or mixture of chemicals with shared metabolic pathways.

In keeping with the committee charge, this chapter discusses key scientific issues regarding approaches for pharmacokinetic modeling of trichloroethylene based on existing metabolic information and uses of pharmacokinetic modeling results for trichloroethylene risk assessment. Discussion of approaches to pharmacokinetic modeling of trichloroethylene focuses on (1) the relative strengths and weaknesses of different model structures and parameterization, including the tradeoff between model complexity (and hence completeness) and uncertainty, and (2) the evaluation of model uncertainties. Discussion of the uses of pharmacokinetic modeling results for risk assessment of trichloroethylene focuses on (1) dose metrics for developing human equivalent doses, route-to-route extrapolation, and use in biologically based dose-response modeling; and (2) uncertainties associated with pharmacokinetic-based dose metrics and consideration of non-pharmacokinetic-based scaling approaches.

This chapter does not include an exhaustive review of the literature on pharmacokinetic models for trichloroethylene. The pharmacokinetic models used in the U.S. Environmental Protection Agency (EPA 2001b) draft health risk assessment of trichloroethylene, several of the pharmacokinetic models published since that assessment, and a model later commissioned by EPA and the U.S. Air Force (USAF) to deal with some problems of the earlier health risk assessment are the focus of this chapter.

OVERVIEW OF PHARMACOKINETIC MODELS

Pharmacokinetic models mathematically describe the absorption, distribution, metabolism, and elimination of a chemical in an organism as a function of time. Similar descriptions for metabolites also can be incorporated into pharmacokinetic models for the parent compound. Pharmacokinetic models typically include compartments that represent specific organs and tissues as well as lumped tissue compartments and are represented by using systems of differential equations. Whether a specific tissue compartment is included in a pharmacokinetic model depends on how involved that tissue is in disposing of the compound (e.g., portals of entry or excretion, sites of metabolism, targets of toxicity) and on its utility as a biomarker of exposure or response. Pharmacokinetic model development is an iterative process; the mathematical model is used to simulate data and the simulated data are compared with real data to refine the mathematical model. "All models are wrong, but some models are useful" (attributed to G. Box [Kokko 2005]). There will never be a comprehensive model that perfectly describes all the exposure and response relationships for any chemical in laboratory animals or humans, but some models may be adequate for predicting useful internal dose metrics, and some models may provide better predictions than others.

Physiologically based pharmacokinetic (PBPK) models define model parameters in terms of directly interpretable anatomic, physiologic, or biochemical quantities. In a basic PBPK model, the tissue compartments are linked by blood flow and have associated physical volumes and partition coefficients that describe the relative degree to which a given chemical (e.g., trichloroethylene) is soluble in each of those tissues versus blood. Although the fundamental mathematical forms of pharmacokinetic and PBPK models with the same compartments may be identical, parameterization in terms of measurable physiologic quantities introduces several advantages (Gibaldi and Perrier 1982). For example, blood flow rates are well characterized in many species, providing a simple and rational method for adjusting a PBPK model to extrapolate across species (e.g., from laboratory animals to humans). Moreover, direct measurements can be independently obtained for some PBPK parameters, rather than relying solely on the results of dosing experiments.

The complexity of a pharmacokinetic model depends on the availability of data, the certainty and confidence in the scientific understanding of the processes described by the model, and the intended use of the model. In general, one begins with the simplest model that describes the data and adds complexity to the structure based on experimental data, lack of model fit to the data, and lack of model applicability to a specific end point of interest. For example, if one is interested only in evaluating the concentration of the parent compound in blood and other tissues over time, the model structure can be very simple. Disappearance of the parent chemical may be described by a "whole-body" metabolism rate and details on different metabolic pathways are not necessary. Pharmacokinetic models are powerful tools that can be used to identify data gaps and research needs. As mechanistic hypotheses are developed, modifications to the model may be necessary to describe a more appropriate dose metric. For example, if one is interested in looking at the effects of a putative toxic metabolite on a specific organ (e.g., kidney), the model structure will likely be more complex.

To account for total body mass (and volume) and total cardiac output (flow), pharmacokinetic models typically include "lumped" tissue compartments that are not relevant to describe a particular chemical. For example, brain, muscle, and skin are usually not included as discrete compartments unless those tissue concentrations are direct targets for prediction (e.g.,

brain concentration for predicting neurotoxicity). Instead, tissues are lumped as richly (or rapidly) and poorly (or slowly) perfused tissue groups. The richly perfused tissue group typically includes organs and tissues such as the brain, kidney, and alveolar region of the lungs, and the poorly perfused group includes tissues such as muscle and skin.

Advantages and Limitations

PBPK models hold particular promise in assessing human health risks from multiple routes of exposure to the same chemical or from exposures to related chemical mixtures, because of their ability to predict doses at the tissues where relevant toxic effects occur. Traditional metrics such as the lifetime average daily dose fail to reflect differences in metabolism and disposition by exposure route, particularly when metabolic rates are saturable or when multiple exposures occur simultaneously. Theoretically, an accurate PBPK model is ideal for characterizing human health risk from a complex exposure pattern involving chemical mixtures, multiple exposure routes, saturable binding and metabolism, and any other situation resulting in a nonlinear relationship between the exposure metric and the target organ dose.

PBPK models are difficult to develop, limited to predicting concentrations in particular tissues, and imperfectly model the processes their creators seek to describe. Compartments with different characteristics often must be grouped together to make parameterization and analysis feasible. Simplifying assumptions may be made without confirmation to establish parametric differential equations (e.g., the steady-state assumption used to derive the Michaelis-Menten equation, as per Gibaldi and Perrier [1982], or the common assumption of flow-limited exchange). Imperfect models can still be quite useful (Morgan et al. 1990), but model predictions for species or exposure patterns other than those used to develop the model should be interpreted with caution after giving careful attention to model assumptions.

Pharmacokinetic model building is a difficult task, as for any complex inference problem (Neter et al. 1996). Specification of few compartments in a pharmacokinetic model facilitates direct and precise statistical estimation of parameters, provides quick results, and may be sufficient for many purposes. However, oversimplification may lead to a biased prediction. Because health risk assessment is concerned with prediction rather than hypothesis testing or other forms of inference, loss of precision in parameter estimates is acceptable to avoid bias in the prediction. Although specifying many compartments decreases the chance of biased prediction when parameters are estimated solely from dosing data, typical PBPK model parameterization often relies on external estimates in addition to direct fits to dosing data and therefore may not protect so strongly against bias through additional compartments. Bayesian statistical approaches may be particularly advantageous for complex pharmacokinetic systems, allowing one to formally incorporate external information and propagate uncertainties, while relying on experimental data to drive the final parameter estimates toward unbiased values (Wakefield and Rahman 2000).

Flow- Versus Diffusion-Limited Exchange

Flow-limited exchange describes the situation in which a chemical is assumed to always be at dynamic equilibrium between the tissues represented by a compartment and venous blood

leaving those tissues. In this situation, the exchange rate between the blood and the compartment is limited primarily by the blood flow rate to the tissues represented by that compartment. Flow-limited exchange appears to be a default assumption in many PBPK models, but it is sometimes an oversimplification (O'Flaherty 1991). In contrast, diffusion-limited exchange does not solely depend on blood flow rates and may be limited by rates of bone accretion or other processes. Tissues that exhibit diffusion-limited exchange may not be well characterized using a single compartment, in which case they are represented using many compartments connected in layers (O'Flaherty 1991), membrane diffusion models (McCarley and Bunge 2001), or other approaches. PBPK model builders and users should carefully assess default assumptions of flow-limited exchange.

Parameterization of PBPK Models

PBPK models contain two basic parameter types: physiologic and chemical specific. Physiologic parameters describe the organism and include parameters such as body weight, blood flow (total cardiac output as well as flow to different organs and tissues), tissue and organ volumes, and respiratory rates. These parameters are usually specific to a given species and are gleaned from the literature rather than measured. Examples of references from which physiologic parameters for PBPK models are obtained include Brown et al. (1997) and Arms and Travis (1988). Allometric scaling is frequently used in PBPK models for volumes and flows when scaling from animals to humans.

As the name implies, chemical-specific parameters are unique for each chemical and include physicochemical parameters (e.g., tissue partition coefficients) and biochemical parameters (e.g., metabolic rate constants, absorption and excretion rates). Tissue partition coefficients describe the extent to which a chemical is soluble in various fluids and tissues. Ideally, partition coefficients are determined experimentally for each test article and in tissues from each species to be modeled. Partition coefficients for a "typical" tissue may be used as the partition coefficient in a lumped tissue compartment (e.g., liver tissue partition coefficient may be used for the liver and the lumped "richly perfused" tissue compartment). Partition coefficients also can be estimated indirectly by using known chemical properties. For example, Poulin and Krishnan (1996) developed algorithms to deterministically estimate tissue partition coefficients based on the lipid solubility of the chemical and the fat content of the tissue. Tissue partition coefficients also may be estimated based on structurally similar chemicals (or classes of chemicals) (Beliveau et al. 2003). It is possible for partition coefficients for a given chemical to vary with species and even gender within a species. However, in the absence of data to the contrary, it is often assumed that tissue partition coefficients are no species specific (e.g., the partition coefficient in the liver of a mouse is no different from that in a human).

Biochemical parameters also are chemical specific and include parameters such as absorption and excretion rate constants and metabolic rate constants (e.g., first-order rate constant, k; Michaelis-Menten rate constants, K_m and V_{max}). Biochemical parameters may be measured or estimated based on a fit to experimental data. Allometric scaling across species is used to estimate biochemical parameters when data are not available for the species of interest.

Uncertainty

Typical PBPK models include many unknown parameters and often highly multimodal likelihood surfaces, leading to challenging inference problems. In particular, parameter uncertainty can complicate inference. Possible strategies include deterministically fixing underdetermined parameters and restricting parameters to biologically meaningful constraints. Some of these strategies were used in a newly available model discussed below (USAF-EPA 2004a).

Alternatively, one could describe uncertainties in the form of probability distributions on unknown parameters. This leads to an approach known as Bayesian statistical inference. Bayesian statistics approaches inference for a random process by expressing uncertainty about unknown parameters as subjective probabilities. For example, the parameters could be unknown biochemical parameters.

The probability distribution describing uncertainty of the parameters before observing any data is known as the prior probability distribution. After observing data, the prior distribution is updated by using the rules of probability calculus. The updated probability distribution of the parameters is known as the posterior distribution. It contains all relevant information about the unknown parameters. From a Bayesian perspective, all statistical inference can be deduced from the posterior distribution by reporting appropriate summaries. In particular, this includes predictive inference.

In the context of parameterized physical systems, like the PBPK model, posterior inference and simulation for the unknown parameters are also described as the Monte Carlo method to solving the inverse problem (Mosegaard and Tarantola 1995). A recent discussion of this strategy appears in studies of Cornford et al. (2004), Haario et al. (2004), and Robert (2004).

Variability

Uncertainty is distinct from variability inherent to the described process. For example, PBPK models can include subject-specific parameters and describe subject-to-subject variation. This is formalized as a random effects distribution of subject-specific parameters. The variability of this distribution is inherent to the process. Even infinite data would never reduce this variability to zero.

Describing such variability takes the form of a hierarchical extension of the basic model. Let theta (θ) denote the subject-specific parameters, and let $p(y \mid \theta)$ denote the sampling model for the observed data, given the set of PBPK parameters θ. The model is hierarchically extended with a second layer $\theta \sim p(\theta \mid \mu)$ to describe intersubject variability, where mu (μ) represents a set of unknown population parameters. In the context of population pharmacokinetic models, this strategy is described, for example, by Wakefield and Rahman (2000). The general framework is also known as mixed-effects modeling.

TRICHLOROETHYLENE PHARMACOKINETIC MODELS AND RISK ASSESSMENT

A number of pharmacokinetic models for trichloroethylene have been published over the last 30 years. During that time the amount of data from humans and experimental animal models increased significantly and these data improved the scientific understanding of trichloroethylene metabolism and the mode of action of trichloroethylene toxicity, which resulted in increased complexity of the pharmacokinetic models for trichloroethylene and its metabolites.

Exposure to trichloroethylene has been associated with a wide variety of adverse health effects including liver toxicity, kidney toxicity, reproductive and developmental toxicity, neurotoxicity, and immunotoxicity as well as cancer of the liver, kidney, lung, testes, and immune system (lymphoma). Trichloroethylene metabolism is complex (Lash et al. 2000a). As discussed in other chapters, specific metabolites have been causally associated with toxic or carcinogenic responses in different tissues and in different species. There are two major pathways for trichloroethylene metabolism: the oxidative (or cytochrome P-450) pathway and the glutathione-dependent pathway (see also Chapter 1). The flux through these two pathways differs in each tissue and the data suggest that the mode of action, including the putative toxic metabolite (dichloroacetic acid, trichloroacetic acid, chloral, and dichlorovinylcysteine), varies for different end points. To further complicate the picture, trichloroethylene metabolism varies in different species and the mode of action for a given end point also may vary with species (see details in other chapters). Clearly, there is considerable uncertainty and lack of consensus in the scientific community about the mode of action for different end points.

Other factors that complicate the assessment of human health risk from exposure to trichloroethylene include coexposure to other solvents, alcohol consumption, disease states that alter trichloroethylene metabolism and toxicity, interindividual variability in trichloroethylene metabolism, and age. There are also direct and indirect exposures to the putative toxic metabolites of trichloroethylene. For example, dichloroacetic acid and trichloroacetic acid are by-products of water chlorination and are often present in drinking water at very low concentrations, some individuals are directly exposed to chloral via medicinal use, and other parent compounds produce some of the same metabolites as trichloroethylene.

As noted above, trichloroethylene and its metabolites have been associated with toxicity and carcinogenicity in one or more species. The targets of toxicity are not the same for all species and the mode of action for the various toxic end points is not well understood. A comprehensive pharmacokinetic model for use in human health risk assessment would incorporate all potential routes of exposure, target organs, and putative toxic metabolites. Such a model would be unrealistically complex and would require substantial effort to develop and validate. Ideally, the pharmacokinetic model would be linked to a biologically based pharmacodynamic model that describes the mode of action; the linked models would yield a pharmacokinetic-pharmacodynamic model. Because pharmacokinetic-pharmacodynamic models rarely describe all adverse effects, simpler models are developed and iteratively refined to improve their ability to predict human health risk.

The EPA (2001b) draft risk assessment for trichloroethylene included pharmacokinetic models published by Fisher (2000) and Clewell et al. (2000). Since the EPA draft risk assessment was published, EPA and USAF commissioned a work group to develop a "harmonized" pharmacokinetic model for trichloroethylene and its metabolites. The work group comprised scientists from EPA, the USAF, Toxicology Excellence for Risk Assessment (TERA),

and others under contract to the USAF (USAF-EPA 2004a). The work group included Drs. Clewell and Fisher. Other investigators have published pharmacokinetic models for trichloroethylene and metabolites since the 2001 EPA draft risk assessment. Several of the models are discussed below.

Review of Several Trichloroethylene Models

Fisher Models

Fisher (2000) reviewed selected pharmacokinetic models for trichloroethylene in mice and humans, focusing on liver cancer as the outcome of interest for risk assessment. As noted in Chapter 4, trichloroethylene causes liver cancer in mice but not in rats, and trichloroacetic acid is considered the principal metabolite responsible for trichloroethylene-induced liver cancer in mice. Fisher's first-generation model includes a four-compartment description of trichloroethylene disposition (liver, fat, richly perfused, and slowly perfused tissue compartments), saturable oxidative metabolism of trichloroethylene, and a simple one-compartment model for trichloroacetic acid in the liver.

Fisher's second-generation model includes six tissue compartments (a lung compartment was added as it is a target organ in mice, and a kidney compartment was added to describe urinary excretion of trichloroethylene metabolites) and a four-compartment submodel for each trichloroethylene metabolite. The second-generation model for mice included trichloroethylene, chloral hydrate, trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide. In the second-generation model for humans, only trichloroethylene, trichloroacetic acid, and trichloroethanol were described. As in the first-generation models, all metabolism was assumed to occur in the liver for both species in the second-generation model. Neither Fisher model described metabolism via the glutathione pathway. The putative toxic metabolite in the kidney is formed via the glutathione pathway. Because no renal toxicity has been observed in mice, this pathway is not relevant for the mouse pharmacokinetic model. However, it may be important in human kidney toxicity.

Both Fisher models include inhalation and oral exposure to trichloroethylene. Dose metrics in the first-generation model were peak concentrations (C_{max}) and area under the curve (AUC) for trichloroethylene in whole blood and trichloroacetic acid in plasma. Dose metrics in the second-generation model included C_{max} and AUC for trichloroethylene and trichloroacetic acid in whole blood, trichloroethylene and metabolites in tissues, and urinary excretion of trichloroethylene and metabolites.

Clewell Model

The Clewell et al. (2000) pharmacokinetic model structure for trichloroethylene in mice, rats, and humans is much more complex than the Fisher models and includes submodels for metabolites in the three principal target tissues for cancer identified in animal bioassays: lung for chloral, kidney for dichlorovinylcysteine, and liver for chloral, trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide. The model for trichloroethylene includes tracheobronchial, fat, rapidly perfused, slowly perfused, liver, and

gastrointestinal tract compartments. The gastrointestinal tract is composed of the gut lumen, stomach lumen, and gut tissue; this more complex description of the gastrointestinal tract allowed a better fit to experimental data on oral absorption of trichloroethylene in a corn oil vehicle. In addition, the Clewell model links metabolism in tracheobronchial tissue to lung toxicity and metabolism in the liver to liver and kidney toxicity. Like the Fisher models, the Clewell model includes inhalation and oral exposure to trichloroethylene, but the mathematical description of oral absorption is different for the Fisher and Clewell models. The common dose metrics of C_{max} and AUC for trichloroethylene and metabolites in various tissues, as well as urinary excretion, can be calculated with Clewell's model. In addition, the Clewell model includes the ability to calculate the lifetime average daily dose for different metrics (e.g., trichloroacetic acid AUC in liver) and time above a critical concentration for a specific analyte in a specific tissue. Specific dose metrics are discussed for liver cancer (e.g., lifetime average daily dose for trichloroacetic acid AUC and dichloroacetic acid AUC in plasma as a surrogate for liver; C_{max} for trichloroacetic acid and dichloroacetic acid in liver), kidney cancer (lifetime average daily dose for production of reactive metabolites), lung cancer (e.g., lifetime average daily dose for chloral AUC and C_{max} for chloral in the tracheobronchial region), and non-cancer end points.

In contrast to the Fisher models, the Clewell models for rats, mice, and humans include the glutathione pathway of trichloroethylene metabolism. The Clewell model also includes descriptions for metabolites in relevant tissues. Chloral is formed from trichloroethylene and is further metabolized in the lung compartment. 1,2-Dichlorovinylcysteine is formed in the kidney, where it causes cytotoxicity or is further metabolized and excreted in urine. Trichloroethylene undergoes oxidative metabolism in the liver to form chloral, trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide. There is also a description of enterohepatic recirculation of trichloroethanol glucuronide:trichloroethanol.

Hamonized Model

After publication of EPA's draft health risk assessment for trichloroethylene, a joint USAF-EPA (2004a) prepared a harmonized pharmacokinetic model for trichloroethylene and its metabolites in rats, mice, and humans. Before publication, a draft of the harmonized model was reviewed by a panel of expert scientists, whose comments were considered in the final harmonized model (USAF-EPA 2004b). The harmonized model includes a primary model for the parent compound (trichloroethylene), which is very similar in structure to the Clewell model, and a number of submodels for specific tissues (e.g., trancheobronchial and liver compartments) and specific metabolites (trichloroethanol, trichloroethanol glucuronide, trichloroacetic acid, dichloroacetic acid, and 1,2-dichlorovinylcysteine). The parent trichloroethylene model includes tracheobronchial, rapidly perfused, slowly perfused, fat, gastrointestinal tract (including stomach and duodenum for description of trichloroethylene absorption administered by corn oil gavage), and liver tissue compartments. The harmonized model accommodated oral (bolus and drinking water), inhalation, and intravenous routes of trichloroethylene exposure. The model also has the capability to describe fat as a diffusion-limited tissue compartment.

Like the Clewell model, the harmonized model includes metabolism to chloral in the tracheobronchial region, hepatic metabolism of trichloroethylene to trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide, and enterohepatic

recirculation of trichloroethanol glucuronide:trichloroethanol. Unlike the Clewell model, the harmonized model includes hepatic metabolism of trichloroethylene to 1,2-dichlorovinylcysteine and does not include a separate kidney compartment for 1,2-dichlorovinylcysteine. Instead, it is assumed that 1,2-dichlorovinylcysteine is formed in the liver and ends up in the kidney where it can result in toxicity or be further metabolized and excreted in urine as *N*-acetyldichlorovinylcysteine.

Dose metrics in the harmonized model include the concentration of trichloroethylene in blood and tissues, trichloroethylene AUC in blood, instantaneous concentration and AUC for chloral in the tracheobronchial region (dose metric for lung), total amount of trichloroethylene metabolized normalized to body weight (dose metric for metabolism), concentrations and AUC for trichloroacetic acid in plasma and liver (dose metric for liver cancer), concentration and AUC for trichloroethanol in blood (dose metric for non-cancer end points in liver), and total production of a thioacetylating intermediate from 1,2-dichlorovinylcysteine normalized to kidney volume (dose metric for kidney cancer).

Authors of the harmonized model state that it should be useful in risk assessment for end points where the mode of action involves tissue exposure to trichloroethylene, trichloroacetic acid, and trichloroethanol; they acknowledge that other dose metrics (e.g., chloral in lung and 1,2-dichlorovinylcysteine in kidney) are highly uncertain because of a lack of adequate pharmacokinetic data.

Poet Model

None of the above models includes a description of dermal absorption of trichloroethylene. Because trichloroethylene is found in drinking water, there is dermal exposure to trichloroethylene when bathing. It has been shown for other volatile organic compounds in chlorinated drinking water (e.g., chloroform) that dermal absorption occurs in addition to absorption via the respiratory tract when showering (Jo et al. 1990). Poet et al. (2000) incorporated dermal exposure to trichloroethylene in rats and humans and their pharmacokinetic model for trichloroethylene included experimentally determined dermal permeability coefficients for both species. Because humans are exposed to trichloroethylene by the oral, inhalation, and dermal routes, dermal exposure should be included when assessing potential risk from trichloroethylene exposure. Additional data sets in experimental animals and humans after dermal exposure to trichloroethylene may be required.

Albanese Models

Albanese et al. (2002) published a series of models that included different descriptions of the adipose compartment. These models include a standard perfusion-limited compartmental model for adipose, a diffusion-limited model, and a hybrid model with an axial-dispersion model for adipose tissue. However, as noted by the expert reviewers of the harmonized model, it may not be necessary to move away from a diffusion-limited adipose tissue compartment if the model fit to experimental data is not improved (USAF-EPA 2004b). The Albanese paper shows only model simulations of trichloroethylene concentrations in adipose tissue and no comparisons with experimental data.

Simmons Model

Simmons et al. (2002) published a pharmacokinetic model for trichloroethylene in Long-Evans rats that focused on evaluating the neurotoxicity of trichloroethylene. This five-compartment pharmacokinetic model included brain, fat, slowly perfused tissue, rapidly perfused tissue, and liver. Partition coefficients for trichloroethylene in blood, fat, muscle, brain, and liver were determined for the Long-Evans rats. Male rats were exposed to trichloroethylene by inhalation, and blood and tissues were analyzed for trichloroethylene concentrations over time. Gas-uptake studies were conducted and the model was used to optimize V_{max} based on a fit of model simulations for trichloroethylene concentrations in the chamber. The model was then used to simulate blood, liver, brain, and adipose tissue concentrations of trichloroethylene and was compared with observed concentrations of trichloroethylene in those tissues during exposure to trichloroethylene vapors (200-4,000 parts per million). As noted in Chapter 6, trichloroethylene neurotoxicity is attributed to peak trichloroethylene concentrations in brain. This model provides a reasonable fit to the experimental data. If a pharmacokinetic model is to be used to estimate neurotoxicity in humans exposed to trichloroethylene, including a brain compartment is necessary.

Keys 2003 Model

Fisher and colleagues continue to refine earlier versions of their trichloroethylene pharmacokinetic models. An expanded model was published in 2003 (Keys et al. 2003). This model included compartments for lung, heart, brain, kidney, slowly perfused tissue, fat (diffusion limited), rapidly perfused tissue, spleen, gastrointestinal tract, and liver (deep and shallow compartments). The model accommodated oral, inhalation, and intra-arterial exposure and provided for exhalation and metabolism of trichloroethylene. The pharmacokinetics of trichloroethylene in male Sprague-Dawley rats was characterized during and after inhalation exposure to trichloroethylene and after oral or intra-arterial administration of trichloroethylene. Trichloroethylene concentrations in blood and tissues were determined. Trichloroethylene metabolites were neither measured nor modeled. As noted above for the Simmons model, including a brain compartment is advisable if one is to use a pharmacokinetic model to assess neurotoxicity risk from trichloroethylene exposure.

Keys 2004 Model

Dichloroacetic acid is formed ex vivo from trichloroacetic acid (Merdink et al. 1998); hence, the validity of data from early studies in which dichloroacetic acid was measured in animals and people exposed to trichloroethylene has been questioned. The harmonized model has a very simple dichloroacetic acid submodel, in part due to the questioned validity of experimental data on the concentration of dichloroacetic acid in blood and tissues after trichloroethylene exposure. As noted above, there is direct exposure to dichloroacetic acid in drinking water and dichloroacetic acid pharmacokinetics have been studied (Curry et al. 1985, 1991; Gonzalez-Leon et al. 1997, 1999; Saghir and Schultz 2002; Schultz et al. 2002). A pharmacokinetic model of dichloroacetic acid was developed that includes a description of the

ability of dichloroacetic acid to inhibit its own metabolism by suicide inhibition of glutathione *S*-transferase zeta (Keys et al. 2004). Studies were done in animals exposed to dichloroacetic acid as a parent compound rather than as a metabolite of trichloroethylene, which bypasses questions related to ex vivo production of dichloroacetic acid from trichloroacetic acid. Addition of this dichloroacetic acid submodel to the harmonized model will be useful only if experimental data with a high degree of accuracy for blood and tissue dichloroacetic acid concentrations are available.

Dose Metrics

PBPK-based human equivalent doses offer a sensible biologically based approach to adjusting for differences across species but may not improve accuracy if an incorrect dose metric is used. For example, AUC for the target organ concentration as a function of time is a reasonable metric in theory, assuming that the effective damage to the target organ is cumulative and occurs at a rate proportional to the target organ concentration. However, other metrics can be proposed that are just as reasonable, such as the AUC for the log of the target organ concentration. If the toxicologic process leading to tissue damage occurs at a rate proportional to the log concentration, the AUC log concentration would likely be a better measure of exposure. Tissue repair or other compensating mechanisms could suggest alternative metrics, such as an AUC for target organ concentrations exceeding a certain threshold. In practice, it is difficult to know the best metric without experiments designed to compare the predictive ability of different metrics or without understanding the mechanisms of toxicity in detail.

Similarly, for toxicants such as trichloroethylene that have several potentially toxic metabolites, it is difficult to determine which metabolite(s) contributes to any particular health effect. Current dosing experiments are suggestive but were not designed to answer either of these questions. Although PBPK modeling is well motivated and is starting to fill in some gaps in animal-to-human and cross-route extrapolation, trichloroethylene dose-response models based on PBPK modeling are best viewed as plausible, rather than superior models, among many alternatives.

This note of caution is not intended to discourage the continued development and application of PBPK models for trichloroethylene. In fact, the EPA (2001b) risk assessment for trichloroethylene presents a sophisticated and appealing application of PBPK modeling and generally presents those results in an appropriate manner. Researchers should embrace the challenges posed by multiple metabolites and the complexity of the PBPK model predictions, as they suggest a variety of useful experiments with various dose patterns to produce different target organ concentration-time profiles or different ratios of metabolites. Aggressive experimentation in this direction should yield substantial information about the mechanisms of toxicity, best target organ dose metrics, and dose-response relationships for trichloroethylene. Hack et al. (in press) discuss how Bayesian posterior inference in the PBPK model identifies parameters with a high degree of uncertainty and suggest that future kinetic studies be designed to learn about these parameters.

Uncertainty

Hack et al. (in press) describe inference in the harmonized PBPK model (USAF-EPA 2004a), formalized under the Bayesian paradigm by reporting posterior inference. This is a natural and convenient choice for a large hierarchical model of this type (Gelman et al. 1995).

First, the model is extended to a population PBPK model by adding a random effects distribution $p(\theta \mid \mu)$ for subject-specific PBPK parameters θ. Specifically, the population PBPK model is defined by introducing normal and lognormal random effects models $p(\theta \mid \mu)$ for all parameters. The model is completed with conjugate hyperpriors $p(\mu)$. A distinguishing feature of the PBPK model is the physiologic interpretation of the parameters. To ensure meaningful interpretation of the implementation, Hack et al. (in press) restrict parameters to a biologically meaningful domain. This is reasonable and appropriate.

Once the model is specified, estimating the model reduces to inference about the parameters. The use of least squares point estimators is limited by the large number of parameters and small amounts of data. The use of least-squares estimation is reported after imposing constraints for several parameters (Hack et al. in press). This is reasonable for an ad hoc first estimate, but it is important to follow up with a model refinement. This is implemented by Hack et al. by reporting posterior distributions on the unknown parameters. Posterior Markov chain Monte Carlo simulation was used to implement Bayesian posterior inference—again, a natural choice and almost a compulsory consequence of the other two choices (given the difficulties of frequentist estimation in this setting).

The basic idea of Markov chain Monte Carlo simulation is the following. It can be argued that under the Bayesian paradigm most inference takes the mathematical form of expectations of some function of interest with respect to the posterior distribution. For example, a point estimate for a parameter θ is reported as the expectation of θ under the posterior probability model (that is, an integral with respect to the posterior distribution). Similarly, predictive inference can be written as an expectation of the sampling model with respect to the posterior distribution on the unknown parameters. The problem is that these integrals typically are analytically intractable. Markov chain Monte Carlo simulation instead evaluates the desired posterior integrals as sample averages. The sample average is defined as an average over iterations in a computer simulation of a Markov chain that is set up so that the desired posterior distribution is the stationary distribution. Ergodic averages with respect to the simulated Markov chain serve to estimate the posterior integrals. For example, point estimates of parameters are represented as ergodic averages of these parameters over the Markov chain simulation. An important practical advantage of the outlined strategy is the ability to implement inference in nearly any probability model and the possibility to report inference on any event of interest. Markov chain Monte Carlo simulation was introduced by Gelfand and Smith (1990) as a generic tool for posterior inference. See Gilks et al. (1996) for a review.

In the context of PBPK models, the outlined strategy can be carried out as described by Hack et al. (in press). The simulation program MCSim (Bois and Maszle 1997) was used to implement Markov chain Monte Carlo posterior simulation in the extended model. Simulation-based parameter estimation with Markov chain Monte Carlo posterior simulation gives rise to an additional source of uncertainty. Ergodic averages computed from the Markov chain Monte Carlo simulation output represent the desired posterior means only asymptotically, in the limit as the number of iterations goes to infinity. Any implementation needs to include a convergence diagnostic to judge practical convergence. Hack et al. report use of the convergence diagnostic

of Gelman et al. (1996). Although the reported diagnostic statistics are not perfect, the committee finds that they are adequate in light of the highly computation-intensive likelihood. The discussion of model fits and sensitivity of Hack et al. summarizes important features of the posterior inference.

Variability

An important element of variability for the reported risk assessment is the choice of dose metric. The PBPK model provides a comprehensive probabilistic description of all metabolites in all specified compartments. The Markov chain Monte Carlo implementation allows easy inference about any event of interest. In particular, for any tentative dose metric the model includes inference about variation with dose and correlation with other tentative dose metrics. Although the PBPK model cannot deliver a decision on the choice of dose metric, it can simplify the decision by describing the joint distribution of possible dose metrics. The committee recommends that the investigators consider a moderately large set of possible dose metrics, including the metrics described earlier in this chapter, and report the correlation of those metrics over different exposure and inhalation concentrations. Hack et al. (in press) include results on correlation of dose metrics and parameters and suggest that parameters that have little impact on the predicted dose metrics are less critical for risk assessment.

FINDINGS AND RECOMMENDATIONS

EPA's use of the Fisher (2000) and Clewell et al. (2000) PBPK models for trichloroethylene in its 2001 draft risk assessment was reasonable given the available data for liver and kidney cancer. The committee supports the inclusion of multiple dose metrics including AUC, C_{max}, and lifetime average daily dose, as it is not clear which is the most appropriate dose metric for a given end point.

EPA relies on the description by Bois (2000a,b) of uncertainty in the Fisher (2000) and Clewell et al. (2000) models. This includes updating uncertainties by using the paradigm of Bayesian inference and implementation by Markov chain Monte Carlo posterior simulation. Bois' extension to population models captures an important aspect of the variability. A joint probability model for all relevant quantities (concentrations in different tissue compartments) implies a coherent description of the variability across different dose metrics.

None of the currently available PBPK models considers all possible routes of trichloroethylene exposure (e.g., dermal) or dose metrics for all adverse health effects (e.g., neurotoxicity). The harmonized model is a reasonable extension of the Fisher and Clewell models and is a step in the right direction, but the mode of action and appropriate dose metric are not clear for each end point. PBPK models do not resolve the uncertainty about the mode of action, but they can inform experimental designs for studying the mode of action. Moreover, understanding the mode of action drives PBPK model elaboration.

Recommendations:

- Because there is potential for trichloroethylene exposure via dermal absorption, the committee recommends that future PBPK models used for trichloroethylene risk assessment include a description of dermal absorption similar to the approach of Poet et al. (2000).
- The committee recommends additional studies to evaluate how well alternative dose metrics predict toxic response. The model could be used to investigate alternative study designs. For example, one could simulate liver concentrations of trichloroacetic acid in several different groups of laboratory animals that receive the same lifetime average daily dose by different dosing regimens to compare the lifetime average daily dose with an internal dose metric (that is, trichloroacetic acid concentration or AUC in liver). One group of subjects could receive intermittent high exposures to trichloroethylene and another group could receive lower daily doses; some groups may receive the same daily dose by different routes (e.g., inhalation versus drinking water). Carrying out the corresponding studies in laboratory animals would facilitate the desired comparison of alternative metrics with respect to their ability to predict the toxic end point.

The PBPK models used in the 2001 draft risk assessment focused on liver (Fisher) and kidney (Clewell) cancer. End points not addressed by currently available PBPK models include reproductive and developmental toxicity, neurotoxicity, immunotoxicity, and others.

Recommendation: PBPK models should be developed for other toxicity end points, such as neurotoxicity and developmental outcomes.

- Simmons et al. (2002) and Keys et al. (2003) included a brain compartment in their models for trichloroethylene, which could be used to predict target organ doses relevant to neurotoxicity in future generations of PBPK models used for trichloroethylene risk assessment. The committee recognizes that there may be little or no data available to confirm model predictions for brain tissue concentrations of trichloroethylene and metabolites in humans. However, including all relevant uncertainties is key and can be formalized under Bayesian inference and implemented with the Markov chain Monte Carlo approach used by Bois (2000a, b). Description of uncertainties in prior simulation might indicate that the approach is not practical without collecting additional data.
- Fisher and others have incorporated developmental exposure in utero and via lactation in their PBPK models for perchlorate (Clewell et al. 2003a,b; Fisher et al. 2000); this approach could be applied to trichloroethylene to investigate dose metrics relevant to developmental effects of trichloroethylene exposure. See Chapter 9 for additional guidance on producing developmental PBPK models.

None of the PBPK models for trichloroethylene describes the effect of exposure to chemical mixtures that include trichloroethylene. For example, ethanol and trichloroethylene share enzymatic pathways of metabolism.

Recommendation*:* A combined PBPK model for trichloroethylene and ethanol would enable investigation of exposure to this mixture. This approach could be used for other mixtures with shared metabolic pathways or common metabolites. A similar approach could be taken to include the effect of disease states on trichloroethylene disposition (e.g., induction of CYP2E1 in diabetes).

In summary, pharmacokinetic models can be useful tools to identify data gaps and research needs to reduce uncertainty in risk assessment. More data and a better understanding of the mode of action for various end points are needed for a revised trichloroethylene pharmacokinetic model, in conjunction with appropriate pharmacodynamic models, to be useful for further understanding the risks posed by trichloroethylene.

12

Issues in the Assessment of Dose Response

The assessment of dose-response relationships is used to predict the incidence, probability, or magnitude of an adverse health effect in an individual or population for any amount of exposure to a toxicant. Dose-response relationships can also be used to estimate an exposure concentration or range of concentrations likely to correspond to a specific probability or risk of adverse health effects (e.g., dose corresponding to 10^{-6} excess risk of cancer). These assessments should include quantitative descriptions of the uncertainty of those predictions, such as statistical confidence limits or sensitivity analyses in which assumptions used in the analysis are varied. Sensitivity to assumptions is of particular concern with epidemiologic data because of the potential effects of measurement error and uncontrolled confounding (Lash and Fink 2003). The U.S. Environmental Protection Agency (EPA) draft health risk assessment on trichloroethylene used various approaches to assess dose-response relationships for cancer and non-cancer health effects, including point-of-departure methods, linear extrapolation, and nonlinear modeling (EPA 2001b). This chapter discusses those approaches and their application to trichloroethylene.

POINT OF DEPARTURE DETERMINATION

Non-cancer Effects

The point of departure is a dose estimate developed from experimental or observational data on cancer or non-cancer effects. For non-cancer dose-response data, the point of departure has generally been defined as the no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or a modeled dose corresponding to an incremental effect (e.g., the lower 95% limit of the dose or concentration corresponding to a 10% increase in response [LED_{10} or LEC_{10}]). Use of NOAEL and LOAEL has been criticized because of their dependence on features of the experimental design of the study from which they are derived (e.g., spacing of the dose groups) and their lack of consideration of statistical error or the shape of the dose-response curve (Crump 1984). The NOAEL and LOAEL provide only a single summary statistic and are of limited use in describing the quantitative dose-response relationship.

Continuous dose-response models are thus preferred (Faustman and Bartell 1997). However, not all dose-response data sets are suitable for estimating parameters in continuous dose-response models. At least three dose groups are required for continuous dose-response modeling, whereas studies with as few as one or two dose groups can sometimes be used to identify a NOAEL or LOAEL. In its draft risk assessment for trichloroethylene, EPA (2001b) compared a variety of NOAEL, LOAEL, and LED or LEC values calculated from different dose-response data sets and converted to human-equivalent doses by various approaches. Points of departure for inhalation dosing and for oral dosing were then selected from among the lower NOAEL, LOAEL, and LED_{10} or LEC_{10} values for each route of exposure.

The committee found that determining points of departure for non-cancer end points in EPA's draft risk assessment for trichloroethylene was generally consistent with common practice and the dose-response evidence available at the time of the assessment. However, several points should be addressed in the future. First, the criteria used to determine when toxicologic or epidemiologic data are suitable for continuous dose-response modeling should be specified. Second, the rationale for choosing a 10% response level should be provided, and presenting results for several other response levels should be considered. The ability to quantify specific response levels depends on the study design, which often differs in epidemiologic and toxicologic studies. Third, the dose-response model(s) used to estimate LEDs should also be presented. Fourth, the methods used to derive human-equivalent doses from animal data should be described. It is important that the summary statistic used for the conversion (e.g., area under the curve or peak values) be provided and be readily apparent (not placed in footnotes or separate documents). Given the variety of approaches available to derive human-equivalent doses, the results using the different approaches should be presented in tables that allow them to be easily identified and compared. This suggests that multiple dose metrics should be considered for each data set to help inform the selection of the appropriate adjustment methods.

Cancer Effects

For cancer dose-response data, the point of departure is an estimated dose "near the lower end of the observed range without significant extrapolation to lower doses" (EPA 2005a, p. 1-13). Guidance for performing such dose-response assessments is provided in EPA's new cancer guidelines. These guidelines were finalized after the agency conducted the risk assessment on trichloroethylene, so EPA will need to update the assessment of trichloroethylene to ensure that it is consistent with the new guidelines. For example, with the exception of its consideration of kidney cancer, EPA (2001b) proposed the use of only LED_{10} values from rodent carcinogenicity studies (adjusted to achieve human-equivalent doses) as points of departure in the trichloroethylene assessment. The new cancer guidelines now suggest that estimated doses corresponding to a 1%, 5%, and 10% increase in response (LED_{01}, LED_{05}, and LED_{10}) should be presented routinely and considered as potential points of departure and that central estimates as well as lower confidence bounds for estimated doses be presented. It has been reported that the LED_{05} estimate is close to the NOAEL for many conventional bioassays with continuous response variables and that the NOAEL exceeds the LED_{10} estimate for many bioassays with quantal response variables (Allen et al. 1994).

It is important to explain and justify the procedure for selecting the particular response level for the point of departure so that the selection does not seem arbitrary. One procedure for

choosing from among the 1%, 5%, and 10% response levels could be to select the highest response level exceeded by the lowest observed response level among all exposed dose groups. For example, consider a study with doses of 0, 100, 200, and 300 mg/kg/day and observed excess risk of 0%, 8%, 14%, and 20%, respectively. Using the suggested criterion, 5% excess risk would be selected as the response level for the point of departure, as it is the highest among the two options below 8%. A different approach may be necessary when most exposed individuals have unique doses (common in epidemiologic studies). Categorization of exposure in quartiles or other groupings may be helpful in that situation, but the results may be sensitive to the arbitrary cut points used to distinguish categories, just as NOAELs are sensitive to cut points (Bailer et al. 1997), so an explicit procedure should be specified. This procedure should be objective and transparent and should yield a point of departure near the lower end of the range of tested non-zero doses in accordance with EPA guidelines. Other procedures may also be reasonable, so EPA should establish a clear protocol.

Under the current cancer guidelines, a variety of dose-response models may be used to estimate effective doses (EDs) and LEDs from the data. Although the logit and probit models typically used for these estimates should provide similar ED values for any given level of response, their LED values may be more divergent. If the establishment of point-of-departure-based dose-response assessment as a default policy model is intended to avoid the difficulties of choosing from among equally reasonable scientific models, it would be sensible to stipulate a default modeling procedure rather than allowing for a variety of approaches.

The effects of selecting different dose metrics for adjustment to equivalent human doses from animal models may be important for both non-cancer and cancer dose-response modeling. For example, EPA notes that subchronic dosing studies indicate that cumulative exposure metrics may not be appropriate for predicting the risk of liver cancer (EPA 2001b, p. 4-20), but it did not evaluate the fit of cumulative exposure metrics for other end points.

LINEAR EXTRAPOLATION FROM THE POINT OF DEPARTURE TO ZERO DOSE

EPA's cancer guidelines state that "linear extrapolation should be used when there are [mode of action] data to indicate that the dose-response curve is expected to have a linear component below the [point of departure]. Agents that are generally considered to be linear in this range include agents that are DNA-reactive and have direct mutagenic activity, or agents for which human exposures or body burdens are high and near doses associated with key precursor events in the carcinogenic process" (EPA 2005a, p. 3-21). When the mode of action is unclear, EPA suggests that linear extrapolation (or interpolation[1]) be used as a default approach, as it is thought to overestimate the response level for a given dose. EPA guidelines support the presentation of results from more than one approach when alternative models have "significant biological support" or when multiple modes of action appear to exist. In the draft risk assessment on trichloroethylene, the low dose-response function was estimated by extrapolating

[1]The committee used "extrapolation" to describe the process of modeling between the point of departure and zero, because conventionally this term has been applied to the process. However, "interpolation" is a more accurate description when the modeling process is applied to data with a zero dose group. Most toxicologic and epidemiologic data sets include a control group or some observations at zero dose, in which case modeling between the point of departure to zero dose is an interpolation between two points. This is true even after adjusting for background response using excess risk or other risk metrics, although the data point at the origin is sometimes excluded from the dose-response plot (e.g., NRC 1983).

between zero dose and the point of departure. The committee found this approach to be consistent with the current cancer guidelines. Because the mode of action for carcinogenicity is unclear and may include multiple pathways (see Chapters 3 and 4), EPA's presentation of results from both linear and nonlinear model approaches is also appropriate.

The committee recommends that a general study of the implications of linear extrapolation from the point of departure for dose-response assessment be performed in support of all human health risk assessments (not just for trichloroethylene). Such study is warranted because the statistical properties of linear extrapolation between zero dose and the point of departure have never been evaluated, unlike the statistical properties of traditional dose-response modeling techniques such as probit and logit regression (McCullagh and Nelder 1989). Such evaluations typically include mathematical derivations or simulation studies to determine the degree of conservatism compared with hypothetically true dose-response models as well as comparisons among alternative dose-response models using real data sets. If the true shape of the dose-response curve is sigmoidal, the linear extrapolation will likely overestimate the actual risk at a given dose, as suggested by EPA (2005a; p. 3-21), but the validity of that claim and the extent of overestimation are difficult to evaluate without explicitly defining the point-of-departure selection procedure.

Although the linear extrapolation procedure was adopted to avoid the difficulty of choosing from among alternative dose-response models that fit equally well, there appears to be little scientific basis for evaluating its performance. The claim that "the dose-response curve for [trichloroacetic acid] appears linear" (EPA 2001b, p. 4-20) is weak, as it is based on only three data points, two of which appear to fall above the point of departure. The relevant issue is whether the dose-response curve is linear below the point of departure, but there appear to be insufficient data to evaluate this claim for human exposure to trichloroethylene or trichloroacetic acid.

Although linear extrapolation has been advocated as an intentionally conservative approach to protect public health, there are some theoretical reasons to think that sublinear nonthreshold dose-response models may be more relevant for human exposure to toxicants, regardless of the mode of action. One basis for judging that dose-response patterns are not linear is related to how population variability affects the dose-response curve for humans. For example, a possible interpretation of mechanistic data on trichloroethylene for renal cancer is that any individual may have an exposure threshold below which the glutathione conjugation pathway may be less utilized; at an exposure below that threshold, there is possibly no excess risk of an individual developing renal cancer. However, the existence of individual dose-response thresholds does not necessarily imply the existence of a population dose-response threshold below which nobody is at excess risk of renal cancer; in fact, most plausible models for variability in individual dose-response thresholds imply a sigmoidal population dose-response curve even in this case. The flattening and smoothing effects of population variability on the shape of the population's dose-response curve have long been recognized for the deterministic model in which each individual has a tolerance to an exposure and the tolerance values have a Gaussian, logit, or other typical distribution (Dobson 1990), but similar results hold for many alternative models. The discussion above does not account for measurement error, which can "linearize" nonlinear dose-response relationships.

To understand this, consider a general function, $\pi_i(d)$, describing the probability of a specific toxic response in an individual, i, given a dose, d. The probability of response in an

individual randomly selected from a population of n individuals is then given by $\sum \pi_i(d)/n$. The classic tolerance model may then be expressed as:

$$\pi_i(d) = 0, \text{ if } d < \theta_i$$
$$\pi_i(d) = 1, \text{ if } d > \theta_i$$

where θ_i is the tolerance for individual *i*. Although this model describes a dose-response threshold for any individual, the shape of the dose-response curve for a population of individuals is described by the cumulative distribution of values for θ_i. For example, it is well known that a Gaussian distribution for θ produces a probit dose-response model for a population, and a logit distribution for θ produces a logistic population dose-response model (Dobson 1990). One might expect these individual tolerances to vary extensively in humans depending on genetics, coincident exposures, nutritional status, and various other susceptibility factors, producing a continuous distribution with one or more modes and relatively narrow tails describing the population extremes. In contrast, a uniform distribution of tolerance values is required to produce a linear dose response under this model. Under realistic assumptions, the dose-response curve is sublinear below the 10% response level, with only approximate linearity at extremely low doses.

Consider a more complicated model that allows for increasing risk with exposure above the individual threshold in an approximately linear fashion, as one might posit for a mode of action that takes effect only at higher doses:

$$\pi_i(d) = 0, \text{ if } d < \theta_i$$
$$\pi_i(d) = 1 - \exp[-\beta(d - \theta_i)] \text{ if } d > \theta_i$$

where β represents the effect of exposure about an individual's threshold. Although this function produces a classic "hockey-stick" dose-response shape for any individual (Figure 12-1), the same model produces a sigmoidal population dose-response curve with no threshold, assuming among the exposed population a Gaussian distribution for θ (Figure 12-2). Although these dose-response models are just two simple examples, a similar phenomenon of translating individual dose-response functions to population dose-response functions should be considered for any human dose-response assessment. It is important to emphasize that it is the population-based dose-response relationships that are generally observable, not individual dose-response relationships, and population dose-response functions form the basis for public health interventions and regulations. The population dose-response model may take various forms depending on the mode of action and distribution of susceptibility factors among individuals, but both linear dose-response relationships and population thresholds are difficult to derive without resorting to uniform distributions of individual susceptibility factors such as θ.

There is epidemiologic evidence for some toxicants (other than trichloroethylene) suggesting a linear or even a supralinear dose response at low doses in humans (Stayner et al. 2003). Although these data may simply reflect unusual mechanisms or heavy-tailed population distributions of susceptibility, the effects of exposure measurement error can distort the apparent shape of an observed dose-response curve. This is an important difference between epidemiologic and toxicologic studies; the latter tend to have relatively little exposure measurement error because of intentionally administered doses are used. An additional issue that can affect the shape of an agent's dose response curve is background effect from spontaneous

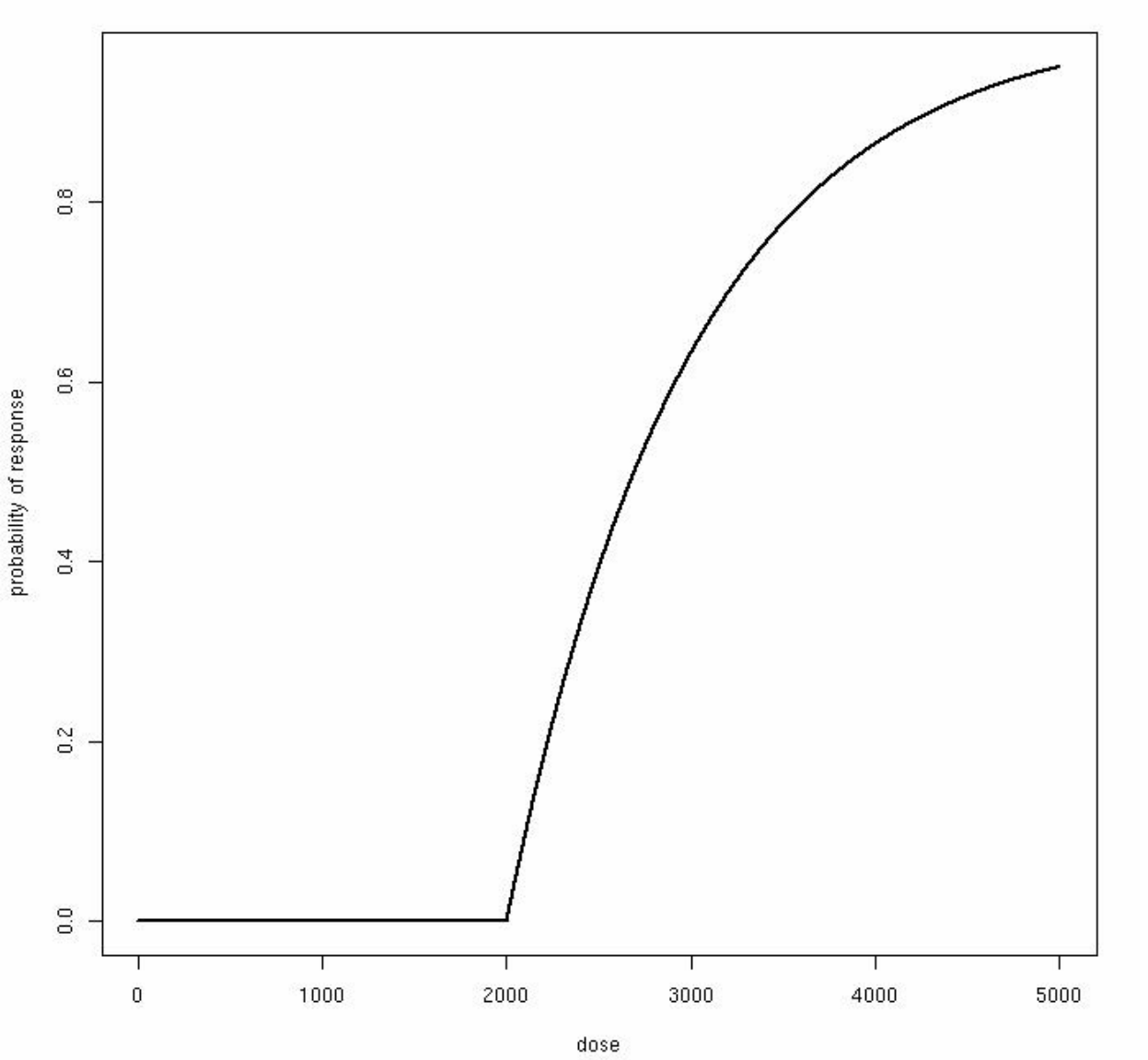

FIGURE 12-1 Classic "hockey-stick" dose-response shape.

occurrence or exposure to other chemicals acting by the same mode of action. If background effects can be assumed to be additive in a mechanistic manner, it would shift the dose-response curve so that response to any additional exposure is linear (Peto 1978; Hoel 1980; Crump et al. 1976; Lutz 1990; Clewell and Crump 2005).

This discussion illustrates an important fact: population variability is an inherent feature of the dose-response curve. Moreover, variability in one parameter could affect the shape of the dose-response curve differently than variability in another parameter (e.g., θ versus β), depending on the underlying probability function $\pi_i(d)$ as well as the shape and location of each population distribution. Therefore, it is difficult to draw conclusions about the shape of the dose-response model from the mode of action alone, without any information on response variability among humans. In fact, any monotonic dose-response model, including the linearized multistage

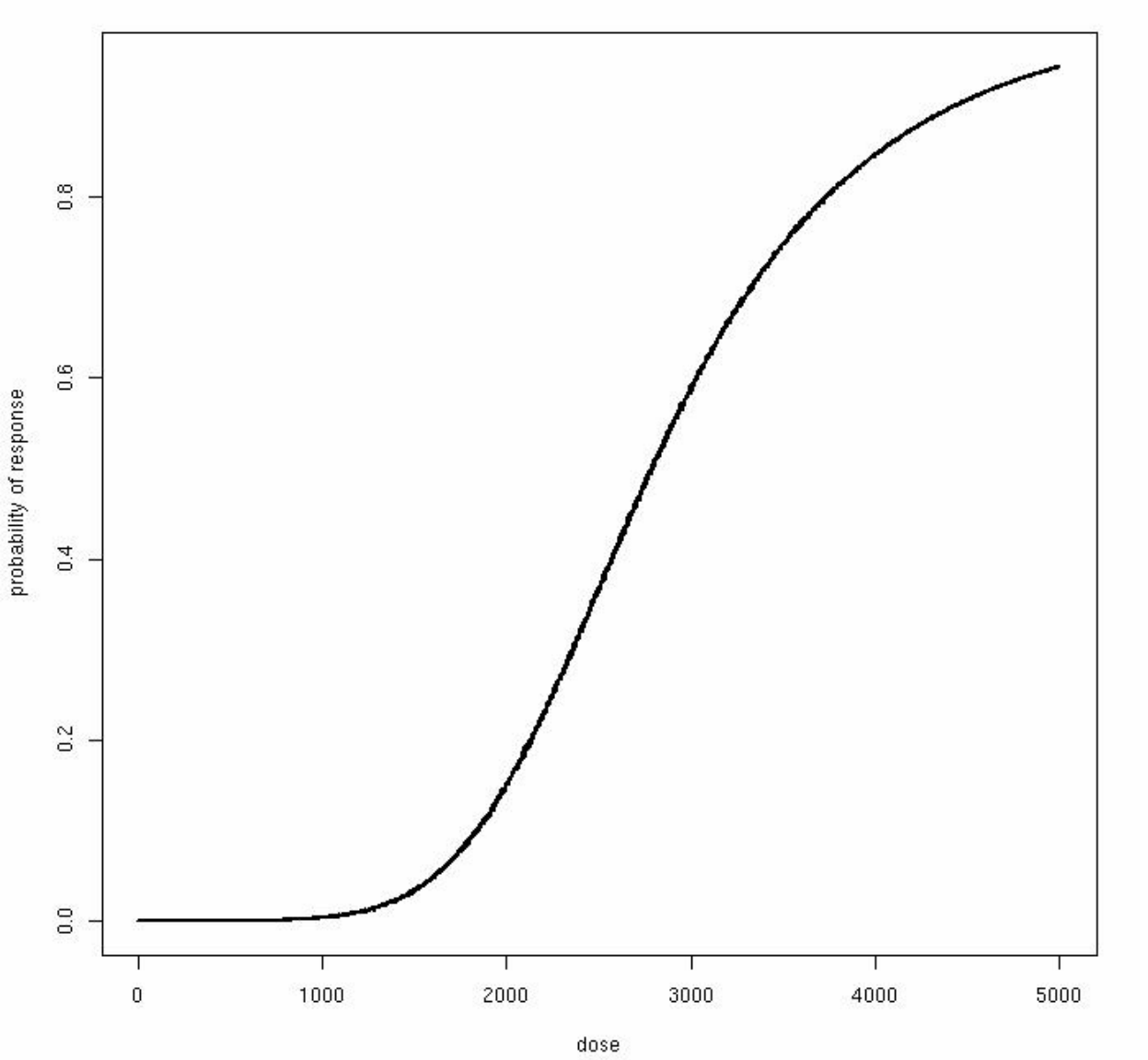

FIGURE 12-2 Sigmoidal population dose-response curve with no threshold.

model, can be defined solely in terms of a tolerance distribution without resorting to mechanistic arguments. These considerations suggest that one must consider both the role of mode of action and the role of response variability among humans in determining the likely shape of the dose-response function.

From a scientific perspective, one approach to characterizing dose-response relationships is to develop models that explain the variability in the available data and, when possible, incorporate known mechanisms of toxicity. Population variability can be directly incorporated within these models by using hierarchical model structures (Allen et al. 1994; Leroux et al. 1996; Gelman et al. 2004; Longnecker et al. 2005) instead of arbitrary uncertainty factors. Although direct measurements of population variation in human susceptibility are rarely available, the relevant parameter(s) could be statistically estimated along with any other parameters in the dose-response model. Alternatively, a surrogate such as variation of rates in a key

toxicodynamic step could be used to estimate population variation in susceptibility. Formal Bayesian methods similar to those applied for physiologically based pharmacokinetic modeling of trichloroethylene offer a natural unified framework for addressing population variability and uncertainty in dose-response assessment and for incorporating information from multiple sources (see Chapter 11). Explicit modeling approaches eventually might replace post hoc applications of uncertainty factors for both cancer and non-cancer dose-response assessment.

From a public health perspective, the optimal dose-response model for any toxicant is often unclear, requiring the judicious use of plausible models that adequately protect health. Moreover, typical toxicologic and epidemiologic data rarely provide confirmation for potentially susceptible subpopulations, such as children, the infirm, and other subgroups, suggesting that, in the face of uncertainty, appropriate correction factors should be applied to protect the population from unnecessary risks.

ALTERNATIVE DOSE-RESPONSE FUNCTIONS

A number of alternatives to point-of-departure-based approaches are also presented for cancer end points in EPA's draft health risk assessment for trichloroethylene, including mechanistic models and linear models for several epidemiologic data sets. The linear models are cursory and in some cases could be improved with more realistic dose-response models, given the original study data. However, exposure ascertainment is weak in many of the epidemiologic studies, as discussed in Chapter 2 and in the EPA assessment, so it may not be worthwhile to conduct more detailed dose-response modeling for many of these data sets.

The committee endorses the general use of epidemiologic data in dose-response assessment but notes that the exposure assessments in most studies may not be of sufficient quality to use for these purposes. Committee members agree that epidemiologic data for trichloroethylene should be evaluated and described more fully than was done in the EPA (2001b) draft risk assessment, giving more weight to data sets of higher quality in the overall evaluation (see Chapter 2). The relative merits and modeling assumptions used in each epidemiologic dose-response assessment should also be clearly delineated; in some cases, it was difficult for committee members to understand how particular epidemiologic data sets were used for dose-response modeling of trichloroethylene.

The committee also endorses EPA's exploration of hypothetical mechanism-based models such as the two-stage cancer model. However, for current pharmacokinetic models for trichloroethylene, the two-stage model is not well validated and should be viewed only as a plausible alternative to other nonlinear dose-response models. Parameterization of mechanistic models is often difficult, and it is important to fully describe the details of the model.

OTHER ISSUES

Definitions of empirical dose-response models, benchmark dose models, and mechanism-based dose-response models given in the EPA draft risk assessment (p. 4-01) are oversimplified. The report groups empirical dose-response models with benchmark dose models and draws too fine a distinction between those models and mechanism-based dose-response models. Although this may seem to be a minor point, it suggests that little or no consideration was given to

approaches that combine mechanism-based model structures with empirical estimation. Any dose-response model can be used to estimate a benchmark dose, even if it is mechanism based. Moreover, mechanism-based models can be parameterized with experimental measurements of individual parameters, "curve-fitting" (that is, statistical estimation using empirical dose-response data), Bayesian analysis synthesizing experimental measurements and dose-response data, or by a combination of these approaches (Leroux et al. 1996; Sherman and Portier 1997; Dunson et al. 2004). Finally, even the parameters for simple logit and probit dose-response models used for curve fitting have biological interpretations, albeit limited ones. It may be best to view dose-response model structures as a continuum from less detailed to more detailed biological information and estimation of parameters in models of dose-response relationships as a separate issue that can be tackled through direct measurement of individual parameters, statistical curve fitting, or Bayesian combination of the two approaches.

UNCERTAINTY ANALYSIS

Uncertainty analysis is the process of providing a description of uncertainty surrounding quantitative estimates of risk. The simplest form of uncertainty analysis is to provide a qualitative description of the sources of uncertainty and their potential effects on the risk estimates. Quantitative assessments of uncertainty can be provided by techniques such as interval analysis and probabilistic analysis. These techniques attempt to predict a range and likelihood of plausible risk estimates rather than a single estimate of the magnitude of risk. In January 2006, the Office of Management and Budget (OMB) released a proposed risk assessment bulletin that states that

> When a quantitative characterization of risk is made available, this should include a range of plausible risk estimates, including central estimates. A "central estimate" of risk is the mean or average of the distribution; or a number which contains multiple estimates of risk based on different assumptions, weighted by their relative plausibility; or any estimate judged to be most representative of the distribution. The central estimate should neither understate nor overstate the risk, but rather, should provide the risk manager and the public with the expected risk.

Although formal quantitative uncertainty analysis techniques are commonly applied in the exposure assessment and pharmacokinetic modeling portions of environmental risk assessment, they are not yet widely used for dose-response modeling (Presidential/Congressional Commission on Risk Assessment and Risk Management 1997). Such applications are substantially different than traditional regulatory approaches such as the application of safety/uncertainty factors and intentionally conservative assumptions such as upper bound dose-response estimates. The proposed OMB bulletin suggests that formal quantitative approaches will be applied routinely in future EPA's risk assessments, including revisions to EPA's trichloroethylene assessment. Below the committee uses the review by Bartell (2005) to summarize some of the quantitative techniques for performing uncertainty analyses.

Interval Analysis

Interval analysis involves estimating the risk twice, using best-case and worst-case scenarios to identify a range (Alefeld and Herzberger 1983; Ferson 1996). While this is a straightforward and easily understood approach, it does not provide information about the relative plausibility of individual risk estimates within the interval. For example, it does not indicate whether each point in the interval is equally likely or whether estimates near the center of the interval are more likely than the estimates near the ends of the interval. Furthermore, single points for base-case and worst-case scenarios may be difficult to define.

Statistical confidence intervals and prediction intervals are another type of interval analysis. These intervals are based on frequentist or Bayesian statistical methods, and attempt to capture the true risk estimate with a fixed confidence level (e.g., 95%) (DeGroot 1989). When traditional frequentist methods are applied, model parameters are usually divided into what is known and unknown, and parameters that are partially understood or for which educated guess may be made are not considered. As an alternative, Bayesian methods offer the advantage of being able to handle such complexities (Greenland 2001).

Probabilistic Analysis

Probabilistic analyses are used to describe risk using one or more probability distributions to indicate the plausibility of an entire range of risk estimates. The most common method is to use Monte Carlo simulation after the initial quantitative risk assessment. The approach involves selecting probability distributions to represent uncertainty in the model parameters. Parameters that are dependent on one another may be specified by such techniques as multivariate distributions, conditional distributions, and rank correlations. Using the specified probability distributions and correlation structure, plausible sets of parameter values are randomly and repeatedly selected. The risk estimates calculated for each set of parameters (tens of thousands or hundreds of thousands) approximate the distribution of uncertainty regarding the risk. Thus, the Monte Carlo distribution is thought to present the range and relative plausibility of various risk estimates. However, cautions have been raised about whether the relative plausibility of an entire range of risk estimates can ever be determined reliably and the possibility of misleading risk mangers (Ferson 1996). Errors in uncertainty propagation can be introduced when correlations between parameters are inadequately characterized or are overlooked.

FINDINGS AND RECOMMENDATIONS

The key scientific issues related to the dose-response assessment for trichloroethylene include selection of the data to be used, selection of the point of departure for low-dose extrapolation, methods for modeling from the point of departure to zero dose, and characterization of uncertainty and variability in estimates of cancer and non-cancer risk.

Although it is preferable to use continuous dose-response models to identify a point of departure for non-cancer risks, the committee recognizes that suitable data on trichloroethylene were not always available for such modeling. Therefore, a NOAEL or LOAEL may be used when a continuous dose-response model cannot be developed to determine LEDs. The selection

of NOAELs and LOAELs is relatively straightforward, but modeled estimates require more explanation and justification.

For dose-response assessment for risks of cancer, EPA's guidelines call for selecting a point of departure from among modeled doses near the lower end of the observed range. A number of response levels and dose metrics are available for performing such assessments, and it is important that all relevant ones are considered and that a clear rationale is provided for selecting the point of departure.

Recommendations:

- Several points of departure should be considered and compared when performing point-of-departure-based dose-response assessments for cancer and non-cancer end points.
- When modeled estimates are used as points of departure in cancer and non-cancer risk assessments, it is important that (1) criteria are established for determining what data sets are suitable for modeling, (2) the selected response level (e.g., 10%) is justified or multiple response levels are modeled and compared, (3) dose-response models are clearly described, (4) different dose metrics are considered and compared to assess whether the choice of metric substantially affects the dose-response assessment, and (5) the methods for estimating human-equivalent doses are specified when animal data are modeled.

There are several approaches to extrapolating from the point of departure to zero, including linear and nonlinear methods. Much emphasis has been given to incorporating mode-of-action information on the carcinogenicity of trichloroethylene into such extrapolations. The committee recommends that information on both the mode of action and on response variability among humans be used to clarify the shape of the low dose-response curve. The mode of action for trichloroethylene as a kidney carcinogen remains unclear and likely involves multiple pathways.

Recommendations: There appear to be insufficient epidemiologic data to support quantitative dose-response modeling for trichloroethylene and cancer. The committee recommends that toxicologic data be used to fit the primary dose-response model(s) and that the available epidemiologic data be used only for validation. The committee does not believe that the available information is sufficient to determine the best dose-response model for trichloroethylene. The default linear extrapolation procedure suggested in the EPA cancer risk assessment guidance can be applied but should first be explicitly defined.

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APPENDIX A

Biographical Information on the Committee on Human Health Risks of Trichloroethylene

ROGENE HENDERSON (*Chair*) is a senior scientist emeritus at the Lovelace Respiratory Research Institute. She is also a clinical professor in the College of Pharmacy at the University of New Mexico in Albuquerque. Her major research interests are in the use of bronchoalveolar lavage fluid analyses to detect and characterize biomarkers of developing lung disease, the toxicokinetics of inhaled vapors and gases, and the use of biological markers of exposure and of effects to link environmental exposure to disease. She has served on a number of scientific advisory boards, including those of the Department of Energy, the U.S. Environmental Protection Agency, the National Institute of Environmental Health Sciences, and the U.S. Army. She was recently appointed chair of EPA's Clean Air Scientific Advisory Committee. Dr. Henderson is a National Associate of the National Academies and is a former member of the Board on Environmental Studies and Toxicology. She received her Ph.D. in chemistry from the University of Texas.

SCOTT BARTELL is assistant professor in the Department of Environmental and Occupational Health and the Department of Epidemiology at the Emory University Rollins School of Public Health. His research interests are in probabilistic models and statistical methods for environmental epidemiology, exposure assessment, risk assessment, and decision analysis. Dr. Bartell was a research scientist at the University of Washington Institute for Risk Analysis and Risk Communication for 4 years, where he conducted research related to child environmental health, chronic beryllium disease susceptibility, applications of toxicokinetic and toxicodynamic models to risk assessment, and other topics. His current research efforts include developing statistical approaches for estimating time-varying exposures using biomarkers, two-stage epidemiologic study design, and applications of toxicokinetic models in epidemiologic analyses involving silica, polychlorinated biphenyls, and methylmercury. Dr. Bartell received an M.S. in environmental health from the University of Washington and an M.S. in statistics and a Ph.D. in epidemiology from the University of California at Davis.

SCOTT BURCHIEL is professor of pharmacology, toxicology, and immunology in the College of Pharmacy at the University of New Mexico. He is also associate dean for research at the college and is director of the New Mexico National Institute of Environmental Health Sciences Center. His research interests are in immunotoxicology, with an emphasis on the effects of drugs and environmental agents on signaling pathways controlling lymphocyte activation and apoptosis,

protooncogene activation, and mechanisms of signaling in human mammary epithelial cells. Dr. Burchiel was a member of the National Research Council Subcommittee on Jet Propulsion Fuel 8. He received his Ph.D. in pharmacology from the University of California at San Francisco.

DEBORAH CORY-SLECHTA is director of the Environmental and Occupational Health Sciences Institute and chair of the Department of Environmental and Occupational Medicine at the University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School. Her research interests are in the relationships between neurotransmitter systems and behavior and how such relationships are altered by exposure to environmental toxicants, particularly the role of environmental neurotoxicants in developmental disabilities and neurodegenerative diseases. Dr. Cory-Slechta has served on numerous national research review and advisory panels, including those for the National Institutes of Health, the U.S. Environmental Protection Agency, and the Centers for Disease Control and Prevention. She is a former member of the National Research Council Board on Environmental Studies and Toxicology's Committee on Toxicology and the Institute of Medicine Committee on Gulf War and Health: Literature Review of Pesticides and Solvents. She received her Ph.D. from the University of Minnesota.

MARY DAVIS is a professor in the Department of Physiology and Pharmacology at the West Virginia University Medical Center. Her research interests are in the toxicology of environmental and occupational pollutants, including water-disinfection by-products, halogenated solvents, and arsenic. She is particularly interested in mechanisms of toxicity in the liver, kidneys, and vascular system. Dr. Davis is a former treasurer of the Society of Toxicology and is a former president of the Society's Allegheny-Erie Regional Chapter. She received her Ph.D. in pharmacology from Michigan State University.

KELLY J. DIX is a scientist in the Toxicology Division of the Lovelace Respiratory Research Institute. She also holds an appointment as clinical assistant professor at the University of New Mexico College of Pharmacy. Her research interests are in the areas of preclinical toxicity and metabolism and the pharmacokinetics of xenobiotics; routes of exposure include inhalation, intratracheal instillation, nasal, oral, intravenous, and dermal. She has been involved in analyzing pharmacokinetic data from preclinical and clinical studies by noncompartmental, classic compartmental, and physiologically based pharmacokinetic methods. Dr. Dix is a diplomate of the American Board of Toxicology. She was a member of the National Research Council Subcommittee on Iodotrifluoromethane. She received her M.S. in environmental sciences and engineering from the University of North Carolina at Chapel Hill and her Ph.D. in toxicology from the North Carolina State University.

MARK GOLDBERG is associate professor at McGill University in the Department of Medicine and associate member in the Department of Epidemiology and Biostatistics, the Department of Occupational Health, the McGill School of Environment, and the Department of Oncology. His research interests are occupational and environmental epidemiology, environmental and occupational causes of cancer, and epidemiologic and biostatistical methodology. Dr. Goldberg was a member of the Institute of Medicine Committee on the Health Effects of the Gulf War that reviewed the literature on pesticides and solvents. He received his M.Sc. and Ph.D. in epidemiology and biostatistics from McGill University.

EVAN KHARASCH is professor and director of the Clinical Research Division of the Department of Anesthesiology at Washington University. His research interests include drug metabolism, mechanisms of anesthetic hepatic and renal toxicity, and interindividual and pharmacogenetic variability in drug disposition and effects. Dr. Kharasch has served on a number of scientific advisory boards, including those of the American Society of Anesthesiologists, the American Society for Pharmacology and Experimental Therapeutics, and the International Society for Anaesthetic Pharmacology. He received his M.D. and Ph.D. in pharmacology from Northwestern University. He is certified by the National Board of Medical Examiners and the American Board of Anesthesiology.

SERRINE S. LAU is professor of pharmacology and toxicology and director of the Southwest Environmental Health Sciences Center at the University of Arizona Health Sciences Center in Tucson. Her research focuses on coupling the metabolic activation of chemicals to their target organ toxicity. Specific areas of interest include genomic and proteomic approaches to understanding the genetic and cellular mechanisms of chemical-induced nephrocarcinogenicity, molecular mechanisms of prostanoid-mediated cytoprotection, and the development of mass spectrometric approaches in proteomics to study chemical-induced alterations in protein structure and function. Dr. Lau completed a term (2002-2004) as councilor of the Society of Toxicology and is currently president of the Society's Mechanisms Specialty Section. She is a former member of the National Research Council Committee on Emerging Issues and Data on Environmental Contaminants. She received her Ph.D. in pharmacology from the University of Michigan.

JOSÉ MANAUTOU is associate professor of toxicology in the Department of Pharmaceutical Sciences at the University of Connecticut. His research interests are in biochemical and molecular mechanisms of hepatotoxicity. Specifically, he is interested in studying the role of multidrug-resistance proteins in the hepatobiliary disposition of xenobiotics. Other areas of investigation include changes in expression of transport proteins in response to chemical liver injury and the hepatoprotective effect of peroxisome proliferators. Dr. Manautou is a councilor of the Society of Toxicology and is a former councilor of the Society's Mechanisms Specialty Section. He was the recipient of the Society's 2006 Achievement Award for significant contributions to the field of toxicology. He received his Ph.D. in pharmacology and toxicology from the Purdue University School of Pharmacy and his postdoctoral training in toxicology at the University of Connecticut.

D. GAIL MCCARVER is associate professor in the Departments of Pediatrics and Pharmacology at the Medical College of Wisconsin. She is also codirector of the Birth Defects Research Center, associate director (training grant and pediatric satellite) of the General Clinical Research Center, and director of the Pediatric Section of Clinical Pharmacology, Pharmacogenetics, and Teratology. She is board certified in pediatrics and neonatal-perinatal medicine. Dr. McCarver's research interests are in genetically determined and environmentally induced differences in xenobiotic metabolism as risk factors for birth defects. Studies involve multiple approaches and tools, including epidemiologic methodology, clinical outcome assessment, molecular techniques, and chemical analysis. She has a grant from the National Institute of Environmental Health Sciences to study trichloroethylene and congenital heart disease. She received her M.D. from the University of Tennessee College of Medicine.

HARIHARA MEHENDALE is professor and Kitty DeGree Endowed Chair in Toxicology at the University of Louisiana at Monroe. His research interests are in pulmonary and hepatic toxicology, mechanisms of toxicology, tissue repair, and toxicology of toxicant mixtures. He is a member of the U.S. Food and Drug Administration Food Advisory Committee and its Dietary Supplements Subcommittee and is chair of the Publications Committee of the American College of Toxicology. Dr. Mehendale received his Ph.D. in physiology from the North Carolina State University. He is a diplomate of the American Board of Toxicology, a fellow of the Academy of Toxicological Sciences, and a former member of the National Research Council Committee on Toxicology.

PETER MUELLER is professor in the Department of Biostatistics at the University of Texas M.D. Anderson Cancer Center. He is also an adjunct professor of statistics at Rice University. His research interests include numerical integration in Bayesian statistics, including Markov chain Monte Carlo methods and pharmacokinetic-pharmacodynamic modeling. He was treasurer of the International Society for Bayesian Analysis and program chair of the American Statistical Association, Section on Bayesian Statistical Science. Dr. Mueller received M.S. degrees in computer science and business from the Technical University, Austria, and in mathematics and physics education from the University of Vienna, Austria; he received his Ph.D. in statistics from Purdue University.

JOHN M. PETERS is Hastings Professor and director of the Division of Occupational and Environmental Health in the Department of Preventive Medicine, Keck School of Medicine, at the University of Southern California. He is also director of the Southern California Environmental Health Sciences Center and holds an appointment as adjunct professor of epidemiology at the University of California at Los Angeles School of Public Health. His research interest is in determining the health effects of environmental exposures using epidemiologic approaches. He has published more than 150 research papers, reports, and chapters on subjects such as the health effects of air pollution, magnetic fields, asbestos, vinyl chloride, and other chemicals in both the workplace and the general environment. Dr. Peters received his M.D. from the University of Utah and his M.P.H. in occupational medicine and Sc.D. in environmental medicine from Harvard University.

THOMAS SMITH is professor of industrial hygiene at the Harvard School of Public Health. His research interests are in characterizing environmental and occupational exposures for studies of health effects and investigating of the relationship between environmental exposure and internal dose. He has developed a toxicokinetic modeling approach for designing exposure evaluations for epidemiologic studies. He is using the approach in a cohort study of lung cancer mortality in the United States trucking industry, where workers are exposed to diesel exhaust. Dr. Smith is also involved in an exposure study of human metabolism of 1,3-butadiene. He was a member of the Institute of Medicine Committee on the Assessment of Wartime Exposures to Herbicides in Vietnam for 7 years. He received his M.P.H. and Ph.D. from the University of Minnesota.

LESLIE STAYNER is professor and director of epidemiology and biostatistics at the University of Illinois School of Public Health. Before joining the university in 2003, Dr. Stayner was chief of the Risk Evaluation Branch of the National Institute for Occupational Safety and Health, Education and Information Division. His research interests include occupational, environmental,

and chronic disease epidemiology; epidemiologic methods; and risk assessment. Recent work has involved using Monte Carlo methods to assess the impact of uncertainties in exposure on analysis of dose-response in epidemiologic data. He received his M.Sc. in epidemiology and occupational health from the Harvard School of Public Health and his Ph.D. in epidemiology from the University of North Carolina at Chapel Hill.

ROCHELLE TYL is director of the Center for Life Sciences and Toxicology at the Research Triangle Institute. Her research interests include reproductive toxicology, reproductive endocrinology, and environmental and comparative toxicology. She has served on numerous scientific committees, including the U.S. Environmental Protection Agency's Endocrine Disrupters Screening and Testing Advisory Committee. Dr. Tyl is a former president of the Teratology Society. She received her Ph.D. in developmental genetics from the University of Connecticut and is a diplomate of the American Board of Toxicology.

JACK VANDEN HEUVEL is professor of molecular toxicology and carcinogenesis in the Department of Veterinary and Biomedical Sciences at Penn State University. He is also codirector of the Center for Excellence in Nutrigenomics. His research interests are in mechanisms of action of hypolipidemic drugs and peroxisome proliferators, steroid hormone-receptor-mediated signal transduction, signal transduction by lipids and fatty acids, and receptor-mediated carcinogenesis. Dr. Vanden Heuvel is also co-owner of Indigo Biosciences, LLC, a contract research company that provides consulting and screening services to pharmaceutical and chemical companies. Some of this work involves evaluating chemicals for their potential to bind with certain enzyme receptors, which are the same targets for trichloroethylene and its metabolites. However, none of his consulting work has been on trichloroethylene or its metabolites. He is also is president of the Molecular Biology Specialty Section of the Society of Toxicology. He received his Ph.D. in environmental toxicology from the University of Wisconsin.

JANICE W. YAGER is senior scientist in the Environment Division of the Electric Power Research Institute. Her current research interests are in applying toxicology to develop biomarkers for human exposure and effects assessment and in the toxicology of metals including arsenic. She has served as an external program peer reviewer of the U.S. Environmental Protection Agency National Health and Environmental Effects Research Carcinogenesis Section, president of the Genetic and Environmental Toxicology Association, and council member of the Environmental Mutagen Society. She has worked on a number of scientific advisory committees including those for the National Institutes of Health, the National Institute of Occupational Safety and Health, the Agency for Toxic Substances and Disease Registry, and the American Conference of Governmental Industrial Hygienists. She serves on the Arsenic Review Panel of the U.S. Environmental Protection Agency Scientific Advisory Board. She received her M.P.H. and Ph.D. in environmental health sciences from the University of California at Berkeley.

APPENDIX B

Participants at Public Sessions

March 23, 2005, Washington, DC

Persons who made formal presentations

Richard Canady, Office of Science and Technology Policy
Weihsueh Chiu, U.S. Environmental Protection Agency
Peter Preuss, U.S. Environmental Protection Agency

Persons who represented sponsoring agencies

Peter Preuss, U.S. Environmental Protection Agency
Sandra Waisley, Department of Energy
Linda Wennerberg, Department of Defense
Richard Wickman, National Aeronautics and Space Administration

Persons who made comments at the open-microphone session

Jim Dix
Paul Dugard, Halogenated Solvents Industry Alliance, Inc.
Debra Hall, Hopewell Junction Citizens for Clean Water
Betty Havel, Village of Endicott Trustee
Sheila Holt-Orstead
Marge Horton
James Little, Resident Action Group of Endicott
Caffey Norman, Patton Boggs
Bernadette Patrick, Citizen Actions to Restore Endicott's Environment
Jennifer Sass, Natural Resources Defense Council
Judy Schreiber, New York State Office of the Attorney General
Lenny Siegel, Center for Public Environmental Oversight
Bob Speigel, Edison Wetlands Association

April 20, 2005, Washington, DC

Persons who made comments at the open-microphone session

Paul Dugard, Halogenated Solvents Industry Alliance, Inc.
J. M. Ensminger
Jennifer Sass, Natural Resources Defense Council

June 9, 2005, Irvine, CA

Persons who made formal presentations

Weihsueh Chiu, U.S. Environmental Protection Agency
Shannon Cunniff, Department of Defense
Christopher DeRosa, Agency for Toxic Substances and Disease Registry
Mustafa Dosemeci, National Cancer Institute
Michael Dourson, Toxicology Excellence for Risk Assessment
Michael Kelsh, Exponent, Inc.
David Mattie, Air Force Research Laboratory
Patricia Stewart, National Cancer Institute

Persons who made comments at the open-microphone session

Cheryl Buchanan
Elizabeth Crawford
Amanda Evans
Neil Fishbein
Leonard Fisher
Jane Horton
Jonathan Parfrey
Lenny Siegel
Christina Walsh

APPENDIX C

Trichloroethylene Metabolism

Knowledge of trichloroethylene metabolism is critical for determining susceptibility, target organ specificity, and gender and species differences and for extrapolating animal data to humans. Lash et al. (2000a) provided a comprehensive overview of the state of knowledge on trichloroethylene metabolism, and key aspects of that review are summarized in this appendix.

Liver is the major site of trichloroethylene metabolism, which occurs mainly through the oxidative and glutathione-dependent pathways. Most of the focus on the oxidative pathway has been on the liver, which has the highest activities of any tissue of the various isoforms of cytochrome P-450s (CYPs). CYP-mediated metabolites of trichloroethylene have been directly associated with liver injury. Trichloroethylene is metabolized primarily by CYP2E1 to a trichloroethylene oxide intermediate, which spontaneously rearranges to chloral. Chloral is further metabolized to trichloroethanol (except in lungs), trichloroethanol glucuronide, and trichloroacetic acid. Minor metabolites include carbon dioxide, dichloroacetic acid, oxalic acid, and *N*-(hydroxyacetyl)aminoethanol. The glutathione-dependent pathway yields glutathione conjugate, *S*-(1,2-dichlorovinyl)glutathione, which occurs predominantly in the liver and can also occur in extrahepatic tissues, but additional biotransformation of *S*-(1,2-dichlorovinyl)glutathione takes place in the kidney. Processing of *S*-(1,2-dichlorovinyl)glutathione through amino-acid-conserving mechanisms yields highly reactive *S*-(1,2-dichlorovinyl)thiol (alpha-chloroenethiolate) through the action of β-lyase and causes renal injury. Other minor metabolites of *S*-(1,2-dichlorovinyl)-L-cysteine may also contribute to renal injury.

ABSORPTION AND DISTRIBUTION

There are three types of exposures to consider for humans and laboratory animals: inhalation, oral, and dermal. Exposure is usually either from trichloroethylene vapor or from trichloroethylene in drinking water. In either form, trichloroethylene is rapidly and extensively absorbed through the lungs and gastrointestinal tract, and less so dermally. Absorbed trichloroethylene is then distributed to different target organs (e.g., lungs, liver, kidneys, nervous system) via the circulatory system. Trichloroethylene readily equilibrates from the circulation into richly perfused tissues, with reported partition coefficients for liver:blood or richly perfused tissue:blood for male rats of approximately 1.2 (Dallas et al. 1991; Fisher et al. 1991). Most trichloroethylene taken into the body is metabolized, but it can also be eliminated via exhalation.

Species differences exist in the fraction of administered dose of trichloroethylene that becomes available for conversion to toxic metabolites in the target organs because of differences in blood flow and overall metabolic rate. For example, blood concentrations of the three metabolites of trichloroethylene—chloral hydrate, trichloroethanol, and trichloroacetic acid—over time after administration of an oral dose of trichloroethylene at 1,000 mg/kg to male Osborne-Mendel rats and male B6C3F$_1$ mice were markedly higher in mice than in rats, whereas concentrations for trichloroethylene were higher in rats than in mice, indicating more rapid metabolism and elimination of trichloroethylene (Prout et al. 1985). Similarly, higher peak plasma concentrations of trichloroacetic acid, the metabolite thought to be primarily responsible for liver effects (Bull 2000), were found in male and female mice than in male and female rats. These observations suggest species differences in susceptibility to the toxic effects of trichloroethylene.

PHARMACOKINETICS

Orally administered trichloroethylene is readily absorbed into the systemic circulation. In rats dosed with [^{36}Cl]trichloroethylene by stomach tube (60 mg/kg), 90% to 95% of the radiolabel was recovered in expired air and urine (Daniel 1963). Administration of a range of doses of labeled trichloroethylene (10-2,000 mg/kg) to rats and mice yielded peak blood concentrations in 1 hour in mice and in 3 hours in rats (Dekant et al. 1984; Prout et al. 1985). Using classic pharmacokinetic analysis, D'Souza et al. (1985) reported that oral and intravenous bioavailability of trichloroethylene was 60% to 90% in nonfasted rats and greater than 90% in fasted rats. Peak blood concentrations occurred between 6 and 10 minutes and blood concentrations were two to three times higher in the fasted rats than in the nonfasted rats. Lee et al. (1996) showed that elimination of low doses of trichloroethylene by metabolism was inversely related to dose and was nonlinear, suggesting that trace amounts of trichloroethylene in the drinking water might not enter the systemic circulation.

Trichloroethylene enters the systemic circulation rapidly after inhalation exposure (Fisher et al. 1991). Peak blood concentrations of trichloroethylene after exposure to trichloroethylene vapors are achieved in 1-2 hours in mice exposed at 100-750 parts per million (ppm), in 4-6 hours in rats exposed at 500-600 ppm, and in 8-12 hours in humans exposed at 100 ppm (Prout et al. 1985; Allen and Fisher 1993). Dermal absorption from exposure to trichloroethylene vapor is negligible, although direct skin contact with trichloroethylene could lead to significant absorption. Dermal absorption of dilute aqueous solutions of trichloroethylene in hairless guinea pigs indicates that significant absorption occurs (Bogen et al. 1992).

Trichloroethylene is metabolized rapidly in the systemic circulation. Physiologically based pharmacokinetic models of rodents and humans show that rodents have a greater capacity to metabolize trichloroethylene than humans (Lash et al. 2000a). Michaelis-Menten affinity constants (K_m) for rodents and humans were estimated to be low (0.25-1.5 mg/L), reflecting a high substrate affinity.

Trichloroacetic acid has a much shorter plasma half-life in rodents than in humans (see Table C-1). The half-life of free trichloroethanol in blood is less than that in plasma for trichloroacetic acid in rodents and humans. The half-life of free trichloroethanol in blood is about 12 hours in humans exposed to trichloroethylene by inhalation (50 ppm) (Muller et al. 1972) and 3 hours in mice after oral intubation (1,200 mg/kg) (Abbas and Fisher 1997). Chloral

TABLE C-1 Plasma Half-life of Trichloroacetic Acid

Route	Administered Dose/Concentration	Species (sex)	Half-Life (h)	Reference
Intravenous injection	5-6 mg/kg TCA	Rat (male)	12	Fisher et al. 1991
		Rat (female)	7	
Intraperitoneal injection	5-10 mg/kg TCA	Mice (male)	7	Fisher et al. 1991
		Mice (female)	3	
Inhalation	42-889 ppm TCA	Mice (male)	16	Fisher et al. 1991
		Mice (female)	7	
	500-600 ppm TCA	Rat (male and female)	15	Fisher et al. 1989
	50 or 100 ppm TCE	Human	86-99	Fisher et al. 1998

Abbreviations: TCA, trichloroacetic acid; TCE, trichloroethylene.

hydrate and trichloroethanol glucuronide are readily measured in the blood of exposed mice (Abbas and Fisher 1997) but not in humans (Fisher et al. 1998).

Pathways of Metabolism

Although many enzymes that catalyze specific steps of the metabolic pathway are widely distributed, a range of activities of specific isozymes are found in different target tissues as well as in a given tissue in males and females from various species.

The first step in trichloroethylene metabolism (Figure C-1) is either conjugation with glutathione or oxidation by CYPs. The oxidative pathway is the major pathway for trichloroethylene metabolism, which occurs primarily in the liver, although different amounts and isoforms of CYP are present in most tissues.

Trichloroethylene oxide metabolite is formed as a result of the action of CYPs, primarily CYP2E1. However, Miller and Guengerich (1982, 1983) and Cai and Guengerich (2001) concluded that trichloroethylene epoxide is not an intermediate in the formation of chloral and chloral hydrate. Therefore, trichloroethylene epoxide cannot be the intermediate responsible for irreversible binding to protein and DNA. Stable lysine adducts were formed in proteins following reaction with trichloroethylene oxide. N(6)-Formyllysine, N(6)-(dichloroacetyl)lysine, and N(6)-glyoxyllysine were formed, with the ratio being influenced by the particular protein (Cai and Guengerich 2000). The majority of the protein adducts (~80%) formed had a collective half-life of only an hour (Cai and Guengerich 2001). These studies also indicate that rat CYP2B1 is more likely to oxidize trichloroethylene to form trichloroethylene oxide and protein lysine adducts than human CYP2E1. The difference is thought to result from the influence of the protein on chloride migration in an enzyme reaction. Trichloroethylene oxide forms adducts with proteins and 2′-deoxyguanosine but not with the other three nucleosides found in DNA (Cai and Guengerich 2001). Approximately 2% of trichloroethylene oxide is adducted with 2′-deoxyguanosine. During the reaction of trichloroethylene oxide with a synthetic 8-mer oligonucleotide, these adducts were short lived having a half-life of only 30 minutes at a of pH 8.5, suggesting the transient nature of these adducts formed from the reaction of trichloroethylene oxide with macromolecules. Green and Prout (1985) also concluded that there was little evidence to support the formation of an epoxide intermediate in the oxidative

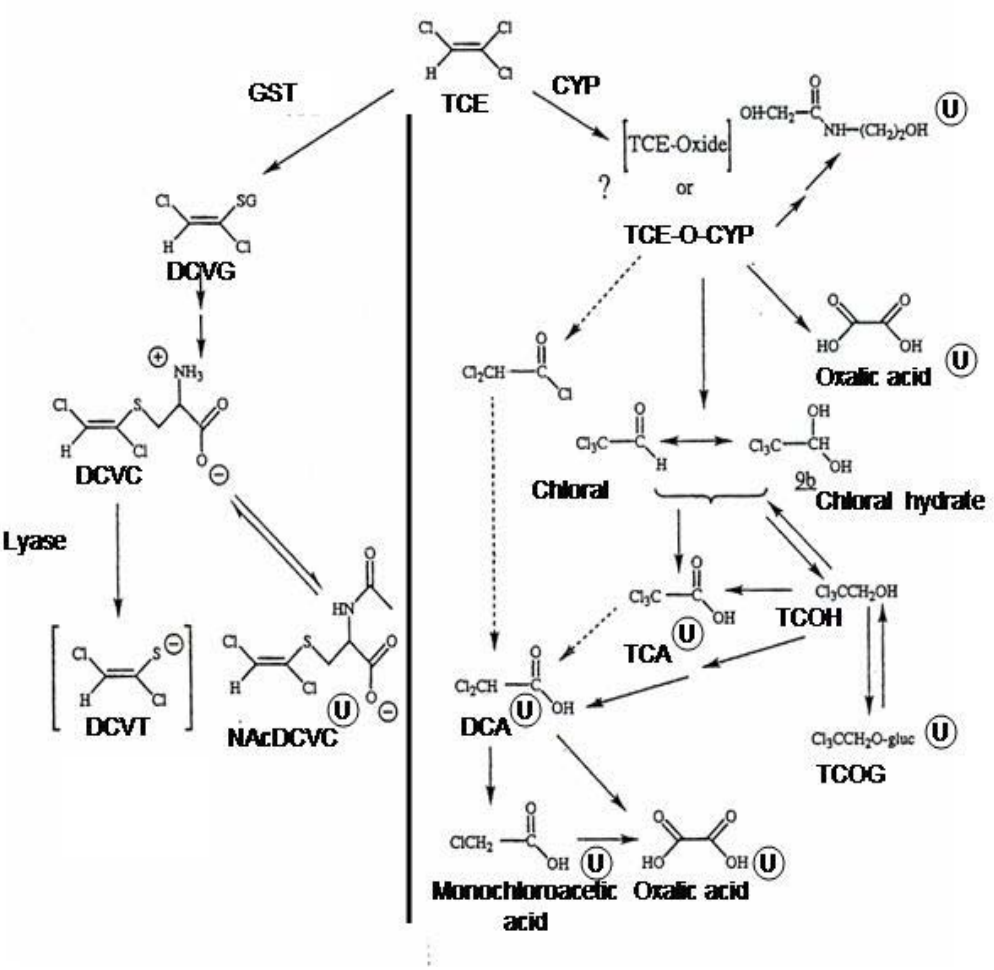

FIGURE C-1 Metabolism of trichloroethylene. Metabolites marked with (U) are known urinary metabolites. Arrows with broken lines indicate other possible steps in forming DCA. Abbreviations: CYP, cytochrome P-450; DCA, dichloroacetic acid; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVT, *S*-(1,2-dichlorovinyl)thiol; GST, glutathione *S*-transferase; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine; TCA, trichloroacetic acid; TCE, trichloroethylene; TCE-O-CYP, trichloroethylene-oxide-cytochrome P-450 complex; TCOH, trichloroethanol; TCOG, trichloroethanol glucuronide.
Source: Adapted from Lash et al. 2000a.

metabolism of trichloroethylene in rat and mouse liver microsomes. However, Forkert and co-workers (Dowsley et al. 1996; Forkert 1999a; Forkert et al. 1999) provided substantial evidence that a compound similar to trichloroethylene, a compound similar to trichloroethylene, 1,1-dichloroethylene, is metabolized by CYPs to an epoxide intermediate. Table C-2 provides K_m and V_{max} values for oxidative metabolism of trichloroethylene in mice, rats, and human microsomal incubations.

Four CYP isoforms are thought to play a role in trichloroethylene metabolism: CYP1A1/2, CYP2B1/2, CYP2C11/6, and CYP2E1 (Guengerich and Shimada1991; Nakajima et

TABLE C-2 Kinetic Constants for Total Oxidative Metabolite Formation from Trichloroethylene[a]

Species	V_{max} (nmol/mg/min)	K_m (mM)	V_{max}/K_m	n
Mouse				
Male	8.60 ± 4.50	0.38 ± 0.41	42.0 ± 28.5	5
Female	26.1 ± 7.3	0.16 ± 0.03	163 ± 37	3
Rat				
Male				
(High affinity)	0.96 ± 0.65	0.072 ± 0.082	23.8 ± 20.6	5
(Low affinity)	2.48 ± 0.97	0.482 ± 0.104	5.3 ± 2.2	
Female				
(High affinity)	2.91 ± 0.71	0.042 ± 0.021	80.0 ± 33.9	3
(Low affinity)	4.31 ± 0.31	0.111 ± 0.027	40.1 ± 7.1	
Human				
Male				
(High affinity)	0.52 ± 0.17	0.012 ± 0.003	48.0 ± 23.1	3
(Low affinity)	0.93 ± 0.17	0.093 ± 0.026	10.7 ± 3.9	
Female				
(High affinity)	0.33 ± 0.15	0.026 ± 0.017	15.3 ± 10.1	3
(Low affinity)	0.72 ± 0.60	0.160 ± 0.162	6.8 ± 5.6	

[a]Oxidative metabolism of trichloroethylene was measured by incubation of microsomes with trichloroethylene at concentrations of 10 μM to 2 mM in the presence of nicotinamide adenine dinucleotide phosphate (2 mM) for 10 min at 37°C and pH 7.4. The following metabolites (assay limits of detection in parentheses) were measured after derivation with pentafluorobenzyl bromide and gas chromatography with electron capture detection: trichloroacetic acid (1 ppm), dichloroacetic acid (0.025 ppm), chloroacetic acid (0.025 ppm), and oxalic acid (10 ppm). Trichloroethanol (0.001 ppm) was detected without any derivatization by gas chromatography with electron capture detection. Chloral hydrate (0.75 ppm) and glyoxylic acid (0.1 ppm) were detected by high-performance liquid chromatography after derivatization with 2,4-dinitrophenyl hydrazine. Data are means ± standard deviation of measurements from the indicated number of experiments.
Source: Adapted from Elfarra et al. 1998. Reprinted with permission; copyright 1998, American Society for Pharmacology and Experimental Therapeutics.

al. 1993; Lash et al. 2000a). CYP2E1 is the major form with the highest affinity for trichloroethylene (Guengerich and Shimada 1991), although the relative roles of the different isoforms can vary depending on physiologic state and the presence of other drugs or inducing agents.

After formation of the trichloroethylene-oxide-CYP, chloral hydrate is the metabolite produced. Because chloral hydrate is rapidly converted to other compounds in the liver, this metabolite is unlikely to be a major contributor to hepatotoxicity or hepatocarcinogenicity. In the lung, however, chloral is the metabolite found, primarily in Clara cells. Oxalic acid, *N*-(hydroxyacetyl)aminoethanol, and dichloroacetic acid also might be formed from the trichloroethylene-oxide-CYP intermediate (Figure C-1). Chloral hydrate is further metabolized to either trichloroethanol or trichloroacetic acid, both of which can be further oxidized to dichloroacetic acid. Some formation of dichloroacetic acid directly from trichloroacetic acid has been observed (Abbas et al. 1996). Trichloroethanol also undergoes glucuronidation to form trichloroethanol glucuronide, which can also undergo enterohepatic recirculation and regenerate trichloroethanol.

Chloral hydrate reduction to trichloroethanol has been reported to be inhibited by ethanol, which suggests that this reaction is catalyzed by alcohol dehydrogenase (Muller et al. 1975;

Larson and Bull 1989). However, Ni et al. (1996) suggested that metabolism of chloral hydrate to trichloroethanol and trichloroacetic acid is catalyzed primarily by CYP2E1. Furthermore, Schultz and Weiner (1979) found that a human lymphoblastoid cell line expressing CYP2E1 metabolized chloral hydrate to mutagenic metabolites, whereas a cell line lacking CYP2E1 expression was inactive in chloral hydrate metabolism (Schultz and Weiner 1979). The proposed pathway for the formation of the mutagenic metabolites appears to be similar in the transfected human lymphoblastoid cell line and in mouse liver microsomes. Both alcohol dehydrogenase and CYP2E1 are likely involved in trichloroethanol formation in mouse liver (Larson and Bull 1989; Lipscomb et al. 1996). Lipscomb et al. (1996) suggested that, at higher substrate concentrations, a lower-affinity enzyme becomes largely responsible for chloral hydrate reduction. However, similar findings have not been demonstrated in rat or human liver cytosol.

Trichloroacetic and Dichloroacetic Acids

Trichloroacetic acid is produced by oxidation of either chloral hydrate or trichloroethanol. Chloral hydrate oxidation is thought to be catalyzed by an aldehyde oxidase, whereas trichloroethanol oxidation is catalyzed predominantly by CYP (Ni et al. 1996). Subsequent reactions of chloral hydrate occur rapidly in the liver, producing trichloroethanol and trichloroacetic acid. This is consistent with trichloroacetic acid being derived from both chloral hydrate and trichloroethanol oxidation (Green and Prout 1985; Dekant et al. 1986b; Larson and Bull 1992a ; Templin et al. 1995).

There are marked differences in chloral hydrate metabolism to trichloroethanol and trichloroacetic acid in liver and blood of rats, mice, and humans (Lipscomb et al. 1996). Kinetic parameters at physiologically obtainable concentrations of chloral hydrate (in the range of 50 μM) showed that chloral hydrate is cleared from human blood through hepatic metabolism at approximately 60% of the rate observed in rodents. Thus, larger amounts of chloral hydrate might be present in human blood and tissues than in rodents after a given exposure to trichloroethylene.

Dichloroacetic acid formation (Figure C-1), particularly in humans, has been a controversial issue. This metabolite was detected in the urine of rats and mice treated with trichloroacetic acid and in blood of mice treated with trichloroethylene (Larson and Bull 1992a,b; Templin et al. 1993). However, problems with analytic methodologies appear to have led to overestimation of dichloroacetic acid formation (Ketcha et al. 1996). It appears that, in the presence of strong acids, some of the trichloroacetic acid in whole blood can undergo nonenzymatic conversion to dichloroacetic acid, leading to overestimation of dichloroacetic acid formation. Templin et al. (1995) found measurable concentrations of dichloroacetic acid in blood from mice but not in rats or dogs. Henderson et al. (1997) identified dichloroacetic acid in children treated therapeutically with chloral hydrate, but whether dichloroacetic acid is formed under nonclinical exposure situations is unclear.

Other Oxidative End Products

Dichloroacetic acid is further metabolized to other species, including oxalic acid, monochloroacetic acid, glycolic acid, and glyoxylic acid (Lash et al. 2000a; Saghir and Schultz

2002). Metabolism (or excretion) of dichloroacetic acid appears to be rapid, as its half-life is much shorter than that of trichloroacetic acid in rats and mice (Larson and Bull 1992a). Degradation of dichloroacetic acid appears to be CYP independent (Tong et al. 1998; Saghir and Schultz 2002). As noted by Lash et al. (2000a), the significance of these metabolites in trichloroethylene-induced toxicity and carcinogenesis is likely to be quantitatively minor.

TISSUE DISTRIBUTION OF OXIDATIVE METABOLISM

The liver is the most important site of oxidative metabolism of trichloroethylene because of its size, abundance of enzymes, and high blood flow. Oxidative metabolism also occurs in the kidneys but at much lower rates than in the liver (Lash et al. 2000a). Interorgan metabolism can affect the biotransformation of trichloroethylene metabolites and target organ toxicity. For example, trichloroethanol glucuronide formed in the liver and excreted into bile can return to the liver by enterohepatic circulation, where it might be hydrolyzed back to trichloroethanol and metabolized to trichloroacetic acid or dichloroacetic acid. Trichloroacetic acid is the major circulating metabolite of trichloroethylene. Concentrations of this metabolite in the blood of mice show a biphasic pattern (Prout et al. 1985), consistent with enterohepatic circulation of trichloroethanol. Renal-hepatic circulation is responsible for excretion of metabolites in the urine, such as trichloroacetic acid and trichloroethanol or trichloroethanol glucuronide.

SEX- AND SPECIES-DEPENDENT OXIDATIVE METABOLISM

Trichloroethylene metabolism differs considerably among species (Lipscomb et al. 1997; Verma and Rana 2003). Blood concentrations of the metabolites trichloroethanol, chloral hydrate, and trichloroacetic acid were found to be severalfold higher in $B6C3F_1$ mice than in Osborne-Mendel rats after acute exposure. Larson and Bull (1992a) reported that metabolism of trichloroethylene to trichloroacetic acid was much higher in mice than in rats, leading to higher blood concentrations of trichloroacetic acid in mice. Rates of oxidative metabolism of trichloroethylene in liver microsomes of male $B6C3F_1$ mice were 2- to 3-fold higher, depending on the dose, than in those from male Wistar rats (Nakajima et al. 1993). Kinetic parameters for total oxidative metabolite formation in liver microsomes from trichloroethylene-treated male $B6C3F_1$ mice, F344 rats, and humans are shown in Table C-2, which indicates that the kinetics are biphasic in liver microsomes from rats and humans but monophasic in liver microsomes from mice. Oxidative metabolism rates were 2- to 2.5-fold faster for high-affinity than for low-affinity processes in rats and humans. Although catalytic rates in humans are lower than in other species, the activity in humans exhibits the same efficiency as that in rats and mice. The rat appears to be a better model than the rabbit for studying human CYP2E1 expression and its role in metabolism of xenobiotics (Wrighton and Stevens 1992; Lipscomb et al. 1998).

Sex-dependent differences in susceptibility to trichloroethylene-induced toxicity and carcinogenicity have been reported, but few studies of metabolic differences between sexes have been conducted. One study that investigated the influence of sex, age, and pregnancy on the expression and regulation of CYP2E1 and CYP2C1I in Wistar rats found no sex-dependent differences but reported a 3-fold decrease in trichloroethylene metabolism to chloral hydrate

between the ages of 3 and 18 weeks and a 2-fold decrease during pregnancy (Nakajima et al. 1992b).

GLUTATHIONE-DEPENDENT METABOLISM

The other possible fate of trichloroethylene besides oxidative metabolism is conjugation with glutathione, which is catalyzed by glutathione *S*-transferases (Figure C-1). Trichloroethylene is metabolized in rats and mice to *S*-(1,2-dichlorovinyl)glutathione both in vivo and in isolated liver microsomes: *S*-(1,2-dichlorovinyl)glutathione appears in bile and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine appears in the urine after exposure to trichloroethylene (Stenner et al. 1997, 1998). Glutathione conjugation of trichloroethylene occurs at rates that are generally severalfold slower than the CYP-catalyzed oxidation reactions. Conversion to the cysteine conjugate is a critical step for subsequent formation of cytotoxic or carcinogenic metabolites.

Metabolism of *S*-(1,2-Dichlorovinyl)glutathione to *S*-(1,2-Dichlorovinyl)-L-cysteine

S-(1,2-Dichlorovinyl)glutathione is metabolized by γ-glutamyltransferase to the cysteinylglycine conjugate *S*-(l,2-dichlorovinyl)- L-cysteinylglycine and then to *S*-(1,2-dichlorovinyl)-L-cysteine (Lash et al. 1988). The requirement of γ-glutamyltransferase for hydrolysis of *S*-(1,2-dichlorovinyl)glutathione was demonstrated with acivicin, a potent and irreversible inhibitor of γ-glutamyltransferase, and with a cosubstrate of the enzyme (Elfarra and Anders 1984; Elfarra et al. 1986). Similarly, inhibitors of dipeptidase and ß-lyase activity prevented or greatly diminished cytotoxicity in vitro or nephrotoxicity in vivo of chemicals that occur before the inhibited step (Elfarra and Anders 1984; Elfarra et al. 1986; Lash and Anders 1986).

S-(1,2-Dichlorovinyl)-L-cysteine is metabolized further by multiple enzymes to yield detoxification products that are excreted and reactive species that are associated with nephrotoxicity and possibly associated with nephrocarcinogenicity.

Metabolism of *S*-(1,2-Dichlorovinyl)-L-cysteine to *N*-Acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine

S-(1,2-Dichlorovinyl)-L-cysteine is *N*-acetylated by a cysteine *S*-conjugate *N*-acetyltransferase to form the mercapturate metabolite *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (Duffel and Jacoby 1982). This metabolite has been detected in the urine of rats (Commandeur and Vemeulen 1990; Larson and Bull 1992a; Birner et al. 1993), mice (Birner et al. 1993), and humans (Commandeur and Vemeulen 1990; Birner et al. 1993: Bruning et al. 1998) exposed to trichloroethylene. The cysteine conjugate *S*-(1,2-dichlorovinyl)-L-cysteine can be regenerated when *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine is deacetylated intracellularly. Thus, the relative rates of *N*-acetylation and deacetylation determine the fraction of the mercapturate that can produce toxic metabolites. In vitro studies indicate that *S*-(1,2-dichlorovinyl)-L-cysteine is rapidly transported into the tubules and is converted to *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine or reactive species covalently bound to cellular proteins (Zhang and Stevens 1989). A larger

proportion of *S*-(1,2-dichlorovinyl)-L-cysteine was bound than *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine, suggesting that deacetylation of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine is relatively slow. Metabolism in the liver also contributes to circulating concentrations of *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine. *S*-(1,2-Dichlorovinyl)glutathione excreted into the bile is converted to *S*-(1,2-dichlorovinyl)-L-cysteine, and some is converted to *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine in the liver and then excreted into the plasma and translocated to the kidneys.

S-(1, 2-Dichlorovinyl)-L-cysteine is metabolized by cytosolic and mitochondrial forms of the enzyme cysteine-S-conjugate β-lyase (Anders et al. 1987; Darnerud et al. 1991; Eyre et al. 1995b; Koechel et al. 1991) (Figure C-1). In the rat kidney, β-lyase exists as a cytosolic glutamine transaminase K isoform (Stevens et al. 1986), while in the liver the kynureninase form of this enzyme is more predominant (Stevens 1985). Similar isoforms of β-lyase have also been reported in mitochondrial fractions of brain tissue (Cooper 2004). Renal metabolism of S-(1, 2-dichlorovinyl)-L-cysteine via cysteine conjugate β-lyase yields a reactive thiol, S-(1, 2-dichlorovinyl)thiol (Figure C-1). This thiol converts to reactive species that alkylate and form covalent cellular nucleophiles, including proteins (Dekant et al. 1988). Net activation rates of trichloroethylene by the β-lyase pathway were greater in mice than in rats (Eyre et al. 1995a,b). The formation of additional products from trichloroethylene *S*-conjugates is described in Chapter 3, including acetylated derivatives and products that are independent of β-lyase activity.

Glutathione-Dependent Metabolism: Tissue, Sex, and Species Differences

The liver and kidneys are the major sites of glutathione conjugation. Because trichloroethylene undergoes glutathione conjugation and is translocated to the kidneys for further metabolism, most glutathione conjugation is thought to occur in the liver. Data on the formation of *S*-(1,2-dichlorovinyl)glutathione from trichloroethylene in liver and kidney cytosol and microsomes of rats and mice are shown in Table C-3. Glutathione conjugation in rats is higher in males than in females. However, measurements of the subcellular fractions found no detectable *S*-(1,2-dichlorovinyl)glutathione in the liver microsomes of male rats but 0.6 nmol per mg of protein in those of female rats (Lash et al. 2000a). In mice, metabolism rates were generally faster in males than in females and were higher than in rats. Higher rates of adduct formation (Eyre et al. 1995a,b) and glutathione conjugation (Lash et al. 2000a) of trichloroethylene occur in the mouse kidney.

HUMAN STUDIES

Metabolism of trichloroethylene appears to be substantially less efficient in humans than in rodents (Lash et al. 2000a). Few of the major metabolites of trichloroethylene have been characterized pharmacokinetically in humans. Fisher et al. (1998) reported that the plasma half-life of trichloroacetic acid in humans ranges from 86 to 99 hours after short-term inhalation of trichloroethylene at 50 or 100 ppm. Oral studies of trichloroethanol (10 mg/kg) and chloral hydrate (15 mg/kg) indicate a plasma half-life of 63 to 65 hours for the metabolite trichloroacetic acid. The plasma half-life for trichloroacetic acid was 51 hours after exposure to trichloroacetic

TABLE C-3 Summary of Metabolism of Trichloroethylene by Glutathione Conjugation in Kidney and Liver Subcellular Fractions from Male and Female F344 Rats and B6C3F$_1$ Mice[a]

Subcellular fractions	*S*-(1,2-Dichlorovinyl)glutathione Formation (nmol/mg of protein or 10^6 cells per 60 min) Male	Female
Rat kidney cells	0.48 ± 0.02	0.65 ± 0.15
Rat hepatocytes	9.70 ± 0.29[b]	2.67 ± 0.69
Rat kidney cytosol	0.45 ± 0.22[c,d]	0.32 ± 0.02[d]
Rat kidney microsomes	ND[b,c]	0.61 ± 0.06
Rat liver cytosol	7.30 ± 2.80[c]	4.86 ± 0.14[d]
Rat liver microsomes	10.3 ± 2.8[c]	7.24 ± 0.24
Mouse kidney cytosol	5.60 ± 0.24[b]	3.70 ± 0.48[d]
Mouse kidney microsomes	5.47 ± 1.41[b]	16.7 ± 4.7
Mouse liver cytosol	24.5 ± 2.4[d]	21.7 ± 0.9
Mouse liver microsomes	40.0 ± 3.1[b]	25.6 ± 0.8

ND, not detectable; limit of detection was 0.05 nmol per mg protein or 10^6 cells.
[a]Results are means ± standard error of measurements from three separate cell or tissue preparations incubated with trichloroethylene at 2 mM, with glutathione at 5 mM for 60 min. *S*-(1,2-Dichlorovinyl)glutathione formation was measured after derivatization of acid extracts with iodoacetate and 1-fluoro-2,4-dinitrobenzene, separation by ion-exchange gradient high-performance liquid chromatography on an amine column using a methanol-acetate mobile phase and detection of *N*-dinitrophenyl *S*-(1,2-dichlorovinyl)glutathione at 365 nm.
[b]Significantly different ($P < 0.05$) from *S*-(1,2-dichlorovinyl)glutathione formation in same species and tissue sample in females.
[c]Significantly different ($P < 0.05$) from *S*-(1,2-dichlorovinyl)glutathione formation in corresponding sample in mice of same sex.
[d]Significantly different ($P < 0.05$) from *S*-(1,2-dichlorovinyl)glutathione formation in microsomes from same sex, species, and tissue.
Source: Lash et al. 1998.

acid alone (3 mg/kg) (Allen and Fisher 1993). Chloral hydrate, oxalic acid, *N*-(hydroxyacetyl)aminoethanol, and dichloroacetic acid have been recovered in the urine of humans exposed to trichloroethylene. The glucuronidation product of trichloroethanol has been recovered in the urine of humans exposed to trichloroethylene. Trichloroethanol glucuronide can undergo enterohepatic recirculation and regenerate trichloroethanol. There are marked differences in chloral hydrate metabolism to trichloroethanol and trichloroacetic acid in human liver and blood (Lipscomb et al. 1996). Chloral hydrate clearance from human blood through hepatic metabolism is 40% lower than in rodents, suggesting that there may be larger amounts of chloral hydrate in human blood and tissues than in those of rodents.

Lipscomb et al. (1997) reported considerable variability in CYP-catalyzed oxidation of trichloroethylene in human liver microsomes. Individual samples seemed to cluster into three groups with K_m values of 16.7 ± 2.5, 30.9 ± 3.3, and 51.1 ± 3.8 μM. K_m and V_{max} values in the groups did not differ among ethnic groups, but the K_m value in females (21.9 ± 3.5 μM) was significantly lower than in males (33.1 ± 3.5 μM).

Table C-4 shows K_m values for CYP1A2, CYP2E1, and CYP3A4, the three isoforms known to catalyze trichloroethylene metabolism in human liver microsomes. CYP1A2 activity was lowest in the low K_m group, and CYP2E1 activity was highest in the high K_m group. There were no significant differences in CYP3A4 activity among the groups (Lash et al. 2000a). CYP2E1 accounts for more than 60% of total microsomal metabolism (Lipscomb et al. 1997,

2003), which indicates that the capacity of humans to metabolize trichloroethylene varies considerably and that factors that alter P-450 activity, particularly CYP2E1 activity, can alter susceptibility to trichloroethylene-induced toxicity. Nakajima et al. (1992b) also reported significant variation in oxidative metabolism of trichloroethylene as a function of physiologic state. The contributions of CYP1A2 and CYP3A4 are low compared with that of CYP2E1 (Shimada et al. 1994).

SPECIES DIFFERENCES

CYP-Dependent Metabolism of Trichloroethylene

In vitro data on metabolite parameters for the oxidative metabolism of trichloroethylene are presented in Table C-5. The data show that humans are less capable than rodents of metabolizing trichloroethylene and chloral hydrate. Formation of chloral hydrate is approximately 20% less in humans than in mice. Dichloroacetic acid formation was not demonstrated in any tissue fraction.

Chloral Hydrate Formation

Humans metabolize trichloroethylene to chloral hydrate at a slower rate than rats or mice (Table C-6) (Lash et al. 2000a). Kinetic parameters in individual human liver samples varied considerably (K_m values of 16-56 μM and V_{max} values of 490-3,455 pmol/min/mg) (Lipscomb et al. 1997). No correlation was found between K_m and V_{max} values among the individual samples.

A concentration-dependent inhibition of CYP2E1 activity was found in human liver microsomes from low, mid, and high K_m individuals. Inhibition paralleled the K_m for trichloroethylene metabolism in microsomal incubations, suggesting that CYP2E1 contributes more to trichloroethylene metabolism in the high K_m sample than in the lower K_m samples (Lash et al. 2000a).

TABLE C-4 Evaluation of Selective CYP-Mediated Metabolism in Human Liver Microsomes Expressing Different K_m Values for Trichloroethylene Metabolism

	CYP Form		
Group	CYP1A2	CYP2E1	CYP3A4
Low K_m	241 ± 186	520 ± 295	2.65 ± 2.66[b]
Mid K_m	545 ± 200	820 ± 372	2.92 ± 2.78[b]
High K_m	806 ± 442	1,317 ± 592	1.78 ± 1.08[b]

Activities of CYP1A2, CYP2E1, and CYP3A4 were measured with phenacetin, chlorzoxazone, and testosterone as substrates, respectively. Data are means ± standard deviation from 10, 9, and 4 samples for the low, mid, and high K_m groups, respectively. Data within each isozyme assay with the same letter are not significantly different from one another ($P < 0.05$) by Kruskal-Wallis one-way analysis of variance.
Source: Lash et al. 2000a.

TABLE C-5 Comparison of Metabolic Parameters for Oxidative Metabolism of Trichloroethylene Determined in Vitro

Metabolic Step	Mouse	Rat	Human
Trichloroethylene to chloral hydrate			
K_m (μM trichloroethylene)	35.4	55.5	24.6
V_{max} (nmol/min/mg of microsomal protein)	5,425	4826	1440
Chloral hydrate to trichloroethanol			
K_m (mM chloral hydrate)	0.12 (low affinity) 0.51 (high affinity)	0.52	1.34
V_{max} (nmol/min/mg of supernatant protein)	6.3 (low affinity) 6.1(high affinity)	24.3	34.7
V_{max}/K_m	52.5 (low affinity) 12.0 (high affinity)	46.7	25.9
Chloral hydrate to trichloroacetic acid			
K_m (mM chloral hydrate)	3.5	6.4	23.9
V_{max} (nmol/min/mg of supernatant protein)	10.6	4.0	65.2
V_{max}/K_m	3.03	0.24	2.73
Dichloroacetic acid degradation			
K_m (mM dichloroacetic acid)	0.350	0.280	0.071
V_{max} (nmol/min/mg of cytosolic protein)	13.1	11.6	0.37
V_{max}/K_m	37.4	41.4	5.2

Source: Lash et al. 2000a.

TABLE C-6 Overall Kinetics of Trichloroethylene Metabolism to Chloral Hydrate and Trichloroethanol

Species	K_m (μM trichloroethylene)	V_{max} (pmol/min/mg)
Rat	55	4,826
Mouse	35	5,425
Human	25	1,440

Metabolism of trichloroethylene to chloral hydrate and trichloroethanol was measured in rat, mouse, and human liver microsomes by gas chromatography. Microsomes were pooled from five rodent and seven human liver samples and were incubated with trichloroethylene at 7.5 to 1,000 μM for 10 (rodent) or 30 (human) min.
Source: Lash et al. 2000a.

Trichloroethanol and Trichloroacetic Acid Formation

The formation of trichloroethanol from chloral hydrate is substantially greater (10- to 200-fold) than the formation of trichloroacetic acid in rodents and humans (Lash et al. 2000a). Formations of trichloroacetic acid in mice and humans were similar, whereas it was approximately 10-fold lower in rats. Thus, trichloroethanol formation will predominate over trichloroacetic acid formation in the liver of these species. It appears that the mice might have two enzymes responsible for forming trichloroethanol from chloral hydrate in liver, whereas rats and humans have a single enzyme. Pravesek et al. (1996) found that at least one mouse cytosolic enzyme responsible for trichloroethanol formation becomes inhibited at high chloral hydrate concentrations.

When hepatic concentrations of chloral hydrate are low (below 0.5 mM), it is preferentially metabolized to trichloroethanol (Sellers et al. 1978; Dekant et al. 1986b; Larson

and Bull 1992a). Lumpkin et al. (2003) found that plasma binding of trichloroacetic acid is higher in humans than in rats and mice. Greater plasma binding in humans would be expected to increase the residence time of trichloroacetic acid but to decrease its availability for hepatic uptake in humans.

Chloral Hydrate Metabolism by Blood

In vitro studies of blood metabolism of chloral hydrate show that more trichloroethanol than trichloroacetic acid is produced (Sellers et al. 1972). More trichloroethanol was produced in rat and mouse blood than in human blood. However, trichloroacetic acid production in lysed human blood was significantly greater than production in rat blood and slightly higher than in mouse blood. Erythrocytes were the site of trichloroacetic acid production. Plasma is the primary site of trichloroethanol production by blood.

Dichloroacetic Acid Metabolism

Dichloroacetic acid is a trichloroethylene metabolite found in rodents but is generally not identifiable in humans. Dichloroacetic acid degradation occurs primarily in rat hepatic cytosol (Lipscomb et al. 1995). K_m values for dichloroacetic acid were 350, 280, and 71 μM and V_{max} values were 13.1, 11.6, and 0.37 nmol/min/mg of protein in mice, rats, and humans, respectively (Lipscomb et al. 1995). Clearance values indicate that degradation of dichloroacetic acid in liver cytosol is less efficient in humans than in rodents.

Glutathione-Dependent Metabolism of Trichloroethylene

Glutathione conjugation of trichloroethylene is much slower than CYP-catalyzed oxidation reactions. Lash et al. (1999) exposed human volunteers (n = 21) to trichloroethylene vapors at 50, 60, or 100 ppm for 4 hours and measured metabolites produced by the glutathione pathway in blood and urine samples. *S*-(1,2-Dichlorovinyl)glutathione was detected in blood within 30 minutes after exposure was completed, and it persisted in the blood for up to 12 hours. Concentrations of *S*-(1,2-dichlorovinyl)glutathione were higher in males than in females. Formation of *S*-(1,2-dichlorovinyl)glutathione is the initial step in the generation of nephrotoxic metabolites and does not directly correlate with it because subsequent detoxification reactions, such as mercapturate formation, can still occur (see Chapter 3 for specific details).

The rate of glutathione conjugation has been found to vary by as much as 3.4-fold in liver cytosol obtained from 9 males and 11 females and by as much as 8.5-fold in microsomes from 5 males and 15 females (Lipscomb et al. 2003). No significant sex-dependent differences in mean activity values were found, but the degree of variation was greater in microsomes from females (7.4-fold) than in males (4.1-fold). Although the overall degree of variation in glutathione *S*-transferase activity is significantly less than that observed with P-450 activity, it could be another factor to account for in a human health risk assessment of trichloroethylene.

APPENDIX D

Exposure Analysis of Selected Studies

When evaluating epidemiologic data for risk assessment, it is important to conduct a meta-analysis of exposures for each study (see discussion in Chapter 2). This is especially important because there are often studies of exposure in an industry, geographic area, or community that can be used together to better understand the nature of exposures during the epidemiologic studies. The studies of trichloroethylene exposures conducted in cardboard manufacturing and small metal cleaning shops in the Ansberg region of Germany provide an excellent example.

COHORT STUDIES

Available studies of trichloroethylene should be rated by type, likely errors, and suitability quantification of dose-risk relationships.

Studies of Aircraft Workers

Six papers reported on cohort studies of aircraft maintenance and manufacturing workers in large facilities.

Spirtas et al. (1991) and Blair et al. (1998)

These studies were conducted on the same maintenance worker cohort at Hill Air Force Base in Utah. Stewart et al. (1998) conducted a detailed exposure assessment. Their work was limited by problems linking subjects with exposures principally because solvent exposures were associated with work in "shops," but work records listed only broad job titles and administrative units. As a result, exposures were probably substantially misclassified. Trichloroethylene was used principally for vapor degreasing and hand cleaning in some areas during 1955-1968. The investigators determined that 32% had "frequent" exposures to peak concentrations (one or two daily peaks of about 15 minutes to trichloroethylene at 200-600 parts per million [ppm]) during vapor degreasing. Work areas were located in very large buildings with few internal partitions, which aided dispersion of trichloroethylene. (This is different from the Henschler et al. [1995] and Vamvakas et al. [1998] studies, which generally had small enclosed work areas.) However,

only a small number of subjects with "high" exposure had long-duration exposures, no more than 16%. Additionally, few workers were exposed only to trichloroethylene; most had mixed exposures to other chlorinated and nonchlorinated solvents. Nonetheless, these modest exposures were associated with some findings suggestive of increases in liver and biliary cancer, multiple myeloma, and non-Hodgkin's lymphoma that were consistent with studies in animals.

Conclusion: A strong exposure assessment was performed, but precision in the exposure assignments was limited by the company's vague personnel data. The cohort had a modest number of highly exposed (about100 ppm) subjects, but overall most were exposed to low concentrations (about 10 ppm) of trichloroethylene.

Garabrant et al. (1988)

This study reported on the overall mortality of a cohort of workers in the aircraft manufacturing industry in southern California. The only exposure metric was years of work. An estimated 37% of the cohort was estimated to be potentially exposed to trichloroethylene, but no information was presented on how they were exposed. Given the enormous misclassification on exposure, the effect of exposure would have to be very large to be detected as an overall risk for the population. Negative findings are to be expected.

Conclusion: The exposure assignments were insufficient to define exposures of the cohort and the frequency of exposures was likely low. Therefore, this study was not useful for determining whether trichloroethylene is related to increased disease risk.

Morgan et al. (1998)

This study evaluated a cohort of 20,508 aircraft manufacturing workers in Arizona. The company conducted a limited semiquantitative assessment of exposure based on the judgment of long-term employees. No details were provided on the protocol for processing the jobs in the work histories into job classifications; no examples were provided. Exposure categories were assigned to job classifications: high = worked on degreasers (industrial hygiene reported exposures were >50 ppm); medium = worked near degreasers; and low = work location was away from degreasers but "occasional contact with [trichloroethylene]." There was also a "no exposure" catgory. No data were provided on the frequency of exposure-related tasks. Without more information, it is not possible to determine the quality of some of these assignments. Only the high category is an unambiguous setting. Depending on how the degreasers were operated, which likely changed over time, operator exposure to trichloroethylene might have been substantially greater than 50 ppm. There are too many possible situations in which an exposure category of medium or low might be assigned to determine whether the ranking is useful. Therefore, the medium and low rankings are likely to be highly misclassified. This study had limited ability to detect exposure-related effects.

Conclusion: The development of exposure assignments this study was insufficient to define exposures of the cohort. Therefore, this study was not useful for determining whether trichloroethylene might cause increased risk of disease.

Costa et al. (1989)

A small cohort of 8,626 aircraft manufacturing workers in Italy was studied. No exposure assessment was used.

Conclusion: This study was not useful for determining whether trichloroethylene may cause increased risk of disease.

Cancer Incidence Studies Using Biological Monitoring Databases

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

Anttila et al. (1995)

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

monitoring. Therefore, it is not clear what biases might be present in the data, especially the possibility of undersampling highly exposed workers.

Conclusion: This study had a small cohort drawn from a wide variety of industries, predominantly degreasing and metal cleaning. Exposures to trichloroethylene were generally low, most less than 14 ppm. The maximum values were generally less than 400 ppm. The duration of exposure was uncertain.

Axelson et al. (1994)

This Swedish study evaluated cancer risk in a small cohort of individuals (1,421 males and 249 females), who were monitored for U-TCA as part of a surveillance system by the trichloroethylene producer during 1955 to 1975. Eighty-one percent of the cases had low exposures (<50 mg/L), corresponding to an airborne concentration of trichloroethylene of approximately 20 ppm. There was uncertainty about the beginning and end of exposure. Exposure was assumed to begin with the first urine sample and to end in 1979 (the reason for this date is unclear). Because the investigators did not have job histories, there is considerable uncertainty about the duration of exposure. Most subjects appear to have had short durations of exposure, but these might have been underestimated. Another concern is the sampling strategy. It was not reported how the workers were chosen for monitoring. Therefore, it is not clear what biases could be present in the data, especially the possibility of undersampling highly exposed workers.

Conclusion: This study had a small cohort drawn from a wide variety of industries, predominantly degreasing and metal cleaning. Exposure to trichloroethylene was generally low (most less than 20 ppm). The duration of exposure was uncertain.

Hansen et al. (2001)

This Danish study evaluated cancer risk in a small cohort of individuals (n = 803) who had been monitored for trichloroethylene exposures in a national surveillance program. The retirement and measurement records contained general information about the type of employer and the subject's job. The subjects in this study came predominantly from the iron and metal industry with jobs such as metal-product cleaner. Each subject had 1 to 27 U-TCA measurements, going back to 1947. Using the linear relationship from Ikeda et al. (1972), the historic median exposures estimated from the U-TCA concentrations were rather low: 9 ppm for 1947 to 1964, 5 ppm for 1965 to 1973, 4 ppm for 1974 to 1979, and 0.7 ppm for 1980 to 1989. However, the distributions were highly skewed, with coefficients of variation of 160% to 370% or estimated geometric standard deviations of 1.9 to 3.7. This is clear evidence that, in general, workers in a wide variety of industry and job groups and identified as "exposed" have low exposures.

Conclusions: This study had a small cohort drawn from a wide variety of industries, predominantly degreasing and metal cleaning, who had generally low exposures (most less than 20 ppm).

Studies of Other Cohorts

Sinks et al. (1992)

An epidemiologic study was conducted of renal cancer among paper board printing workers. The process involved producing food containers from waxed paperboard. Exposures were poorly described. Trichloroethylene was mentioned in material-safety data sheets for one or more materials used by the process but no information was provided about where or how the material was used. Some benzidine-containing materials were used in the process as inks for printing colored forms on the food containers. It was not possible to assess the degree of contact with trichloroethylene or the printing inks.

Conclusions: This study was not useful for assessing risks associated with exposures to trichloroethylene.

Raaschou-Nielsen et al. (2003)

A cohort of 40,049 blue-collar workers was drawn from 347 companies with documented trichloroethylene use. A separate exposure assessment was conducted using regulatory agency data from 1947 to 1989 (Raaschou-Nielsen et al. 2002). The percentage of exposed workers was found to decrease as company size increased: 81% for <50 workers, 51% for 50-100 workers, 19% for 100-200 workers, and 10% for >200 workers. About 40% of the workers in the cohort were exposed (working in a room where trichloroethylene was used). Smaller companies had higher exposures. Median exposures to trichloroethylene were 40-60 ppm for the years before 1970, 10-20 ppm for 1970 to 1979, and approximately 4 ppm for 1980 to 1989.

Conclusions: Only a small fraction of the cohort were exposed to trichloroethylene. The highest exposures occurred before 1970, and the iron and metal industry doing degreasing and cleaning with trichloroethylene had the highest exposures, with a median concentration of 60 ppm and a range up to about 600 ppm.

Henschler et al. (1995)

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

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

Conclusion: The subjects in this study clearly had substantial peak exposures to trichloroethylene that exceeded 2,000 ppm and probably sustained long-term exposures greater than 100 ppm, which are unconfounded by concurrent exposures to other chlorinated organic solvents.

CASE-CONTROL STUDIES

Population level job-exposure matrices (JEMs) have been developed for the United States, Canada, United Kingdom, Germany, and some Scandinavian countries. Community level JEMs have been developed for some cities and cancer registries (e.g., Montreal). If investigators do not develop their own local JEM, local variations across broad regions and countries can add misclassification. Consequently, the use of multiple exposure assignment strategies, such as multiple JEMs and self-assessment by questionnaires, will not give the same results when applied to a given population. For example, the study of renal cell cancer and trichloroethylene exposures by Bruning et al. (2003) applied the CAREX database information developed by the International Agency for Research on Cancer and the British JEM develop by Pannett et al. (1985) to assign exposures to jobs in the work histories of a German set of cases and controls. These two schemes were then compared with a self-assessment of exposures obtained from a questionnaire. In this case, the workers had more knowledge of their personal exposures than usual because they had been told or had observed that they were using either trichloroethylene or Perc (tetrachloroethylene) for degreasing. The schemes gave related but different results. Comparisons were difficult because they did not use the same groupings of industries, which would result in differences in misclassification. For example, the most consistent finding for the CAREX data was a significant increase in renal cell cancer for jobs in "land, air, and sea transport" and "cleaning and waste disposal" and no elevation for "iron, steel, and nonferrous industries" and "occupations with contact to metals," which are difficult to interpret.

Pesch et al. (2000a,b) noted several important general points about exposure assessment for case-control studies. First, the chemical information available to many workers is insufficient for valid recall of agent-specific exposures in general population studies. Second, risk cannot be determined for low-prevalence jobs or industries when studying a regional population. Third, associations with exposure can be highly nonlinear because categoric assignments of duration ("short," "medium," "long," or "very long") and intensity ("low," "medium," "high," or "substantial") are arbitrary and do not necessarily represent a linear dose

relationship. Fourth, large numbers of job titles and low prevalence of long-duration, intense exposure within job title groups greatly reduce power. Job tasks using specific materials have a higher specificity for exposure, but data gathering is limited by interviewer knowledge of the exposures. Fifth, duration and cumulative exposure variables do not consider age at first exposure, which also affects cancer risk.

Studies in the Arnsberg Region of Germany

A series of studies (including Henschler et al. [1995]) have been conducted in an area with a long history of trichloroethylene use in several industries. The main importance of these studies is that there is considerable detail on the nature of exposures, which made it possible to estimate the order of magnitude of exposure, even though there were no direct measurements (see Table D-1).

TABLE D-1 Trichloroethylene Exposure Summary for the Arnsberg Area Studies

Study	Peak Exposures	Long-Term Exposures	Notes
Henschler et al. 1995	>2,000 ppm, machine cleaning with neurological symptoms; about 100 ppm continuous cold cleaning	100 ppm 100 ppm	Cherrie et al. (2001) estimates
Vamvakas et al. 1998	400-600 ppm hot cleaning with neurological symptoms	100 ppm	Cherrie et al. (2001) estimates
Bruning et al. 2003	400-600 ppm hot cleaning, with neurological symptoms	100 ppm	

Vamvakas et al. (1998)

In a follow-up to the Henschler et al. (1995) study, a case-control study was conducted in the Arnsberg region of Germany where there has long been a high prevalence of small enterprises manufacturing small metal parts and goods, such as nuts, lamps, screws, and bolts. Exposures to trichloroethylene resulted from dipping metal pieces into vats, with room temperatures up to 60°C, and placing the wet parts on tables to dry. Some work rooms were noted to be small and poorly ventilated. These conditions are likely to result in high inhalation exposure to trichloroethylene (100-500 ppm). Cherrie et al. (2001) estimated the long-term exposures to be approximately 100 ppm.

Some of the cases included in this study were also pending legal compensation. As a result, there had been considerable investigation of the exposure situation by occupational hygienists from the Employer's Liability Insurance Association and occupational physicians, including walk-through visits and interviews of long-term employees. The legal action could introduce a bias, a tendency to overreport some of the subjective reports by the subjects. However, the objective working conditions were assessed by knowledgeable professionals, who corroborated the presence of the poorly controlled hot dip tanks, extensive use of trichloroethylene for all types of cleaning, and the process descriptions.

Conclusion: These workers had substantial, sustained exposures to high concentrations of trichloroethylene at 400-600 ppm during hot dip cleaning and greater than 100 ppm overall.

Cherrie et al. (2001) concluded that the exposures in the aircraft industry evaluated by Stewart et al. (1991) were likely similar to those of Vamvakas et al. (1998). However, Cherrie et al. overlooked the important difference in room size between these settings: the small- and medium-sized businesses would be using much smaller work rooms than the aircraft-hangar-sized work rooms of the aircraft manufacturers, which would allow the local emissions to dissipate to a greater degree than in the German settings. Cherrie et al. focused on the durations of exposure, without also considering the substantial differences in the types of exposures, which would substantially affect exposure intensity and associated symptoms.

Bruning et al. (2003)

This study is a second case-control follow-up of renal cell cancer in the Arnsberg area of Germany, which was intended to deal with some of the methodological issues present in the two earlier studies. The major advantage of studies in the Arnsberg area is the high prevalence of exposure to trichloroethylene because of the large number of companies doing the same kind of industrial work. An interview questionnaire procedure for self-assessment of exposures similar to the one used by Vamvakas et al. (1998) was used to obtain detailed information about solvents used, job tasks, and working conditions, as well as the occurrence of neurological symptoms. The industry and job title information in the subjects' job histories were also analyzed by two schemes of expert-rated exposure assignments for broad groups of jobs. The CAREX database from the European Union and the British JEM developed by Pannett et al. (1985) were used. This was done to obtain a potentially less biased assessment of the exposures. However, both of these rating approaches are very broad and they have potentially high rates of misclassification of exposure intensity in job groupings and industry groupings. High-intensity exposures tend to be a small fraction of any broad grouping with exposure, such as topside workers among all coke oven workers (Lloyd 1971). This dilution of risk increases with the breadth of the group. The substantially increased risk of lung cancer in coke oven workers is nearly undetectable among all steel workers, whose standardized mortality ratio shows only a small elevation (Lloyd and Ciocco 1969).

In an attempt to avoid reporting biases associated with the legal proceeding for compensation, analyses were conducted on self-reported exposure to selected agents (yes or no). The regional use of trichloroethylene and Perc (tetrachloroethylene) were so widespread that most individuals recognized the local abbreviations. If individuals claimed to be exposed when they were not, it would reduce the finding of a relationship if one existed. Similarly, subjects were grouped by frequency of perceived symptoms (any, less than daily, and daily). Overreporting would also introduce misclassification and reduce evidence of any relationship.

Self-reporting of exposure to chemicals in case-control studies is generally considered unreliable because, within the broad population, workers rarely know which specific chemicals they are exposed to. However, in cohort studies and case-control studies in which one industry dominates a local population, this is less likely because the numbers of possible industries and job titles are much smaller than in a broad population. The Arnsberg area studies focused on a small area where one type of industry was very prevalent, and that industry used primarily just two solvents: trichloroethylene and tetrachloroethylene. As a result, it was common knowledge among the workers what solvent an individual was using, and, for most, it was trichloroethylene. If the base population was enlarged to include more areas, which did not have the same focus of

industry, the ability to detect this association would be expected to decrease, as was found in the study by Pesch et al. (2000a), which included the Arnsberg area plus four other areas (reviewed below).

Conclusions: Again, this is the same basic Arnsberg population studied by Vamvakas et al. (1998), so the exposures will be the same: substantial, sustained high exposures to trichloroethylene at 400-600 ppm during hot dip cleaning and greater than 100 ppm overall.

Pesch et al. (2000a)

This was a multicenter study of renal cell carcinoma in Germany, which included the Arnsberg region plus four others. Two general JEMs, British and German, were used to assign exposures based on subjects' job histories reported in an interview. Researchers also asked about job tasks associated with exposure, such as metal degreasing and cleaning, and use of specific agents (organic solvents chlorinated solvents, including specific questions about carbon tetrachloride, trichloroethylene, and tetrachloroethylene). A category of "any use of a solvent" mixes the large number with infrequent slight contact with the few noted earlier who have high-intensity and prolonged contact.

Conclusions: While this case-control study includes the Arnsberg area, several other regions are included as well, where the source of the trichloroethylene and chlorinated solvent exposures are much less well defined. As a result, most subjects identified as exposed to trichloroethylene probably had minimal contact, averaging concentrations of about 10 ppm or less.

Brauch et al. (1999, 2004)

This pair of studies evaluated mutations in the von Hippel-Lindau gene in subjects drawn from the Vamvakas et al. (1998) cohort. The findings were analyzed with the combination-exposure index that uses both exposure duration and severity of neurological symptoms.

Conclusions: This is an appropriate use of the Vamvakas et al. (1998) exposure assignments for the individual cases evaluated. These workers had substantial, sustained high exposures to trichloroethylene at concentrations of 400-600 ppm during hot dip cleaning and greater than100 ppm overall.

APPENDIX E

Peroxisome Proliferators and Liver Cancer

BACKGROUND

Peroxisomes, subcellular organelles found in the cytoplasm of mammalian and other cells, have important metabolic functions, in particular fatty acid oxidation. Several groups in the mid-1960s first reported the phenomenon of peroxisome proliferation when significant increases of hepatic peroxisomes were seen in response to administration of the hypolipidemic drug clofibrate to rats (Duve 196). In addition to peroxisome proliferation and hepatomegaly, peroxisomal fatty acid oxidation is induced, and long term administration of this drug (and similar compounds) causes hepatocarcinogenesis (Reddy et al. 1976). A number of compounds were later identified that share the morphologic and biochemical response of clofibrate and were deemed "peroxisome proliferators" (see Table E-1). Peroxisome proliferators are a diverse group of chemicals that include the fibrate class of hypolipidemic drugs (e.g., clofibrate, ciprofibrate), Wy14,643 (often used as the prototypical peroxisome proliferator), commercially used plasticizers (phthalates, perfluorinated fatty acids), and endogenous fatty acids (see Table E-2).

TABLE E-1 Characteristic Effects of Peroxisome Proliferator Compounds

Morphologic changes	Hepatomegaly
	Increase in number and size of peroxisomes
	Hepatocarcinogenesis (tumor promotion)
Biochemical changes	Decrease in serum lipids, triacylglycerols, and cholesterol
	Peroxisomal protein induction (acyl-CoA oxidase, bi/trifunctional protein, carnitine acetyltransferase)
	Mitochondrial protein induction (acyl-CoA dehydrogenase, carnitine palmitoyl transferase)
	Microsomal protein induction (CYP4A)
	Cytosol protein induction (acyl-CoA hydrolase, malic enzyme, fatty acid binding protein)
Other characteristics of biochemical changes	Species differences: (rat, mouse > hamster, guinea pig > rabbit, dog, monkey)
	Sex differences: (male > female)
	Target organ specificity (liver > kidney, heart, small intestine > other organs)

TABLE E-2 Representative Peroxisome Proliferators

Commercial Category	Compound
Hypolipidemic drug (approved in U.S. and elsewhere)	Gemfibrozil (U.S.)
	Clofibrate (U.S. and others)
	Ciprofibrate (France)
	Fenofibrate (Other countries, not U.S.)
Hypolipidemic drug (not approved)	Wy-14,643
	Nafenopin
	BR-931
	Methylclofenepate
Herbicide	Lactofen
	Fomasafen
	2,4-Dichlorophenoxyacetic acid
	2,4,5-Trichlorophenoxyacetic acid
Plasticizers and polymerizers	Di-(2-ethylhexyl)phthalate
	Di-(2-ethylhexyl)adipate
	Di-*n*-butyl phthalate
	Perfluorooctanoic acid
	Perfluorooctanesulfonate
Solvents	Trichloroethylene
	Perchloroethylene
Miscellaneous pharmaceutical	Valproic acid (antimania, approved U.S. and elsewhere)
	LY-171,883 (leukotriene D4 receptor antagonist [not approved])
	Dehydroepiandrosterone (dietary supplement and human adrenal steroid, approved in U.S. and elsewhere)

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of transcription factors. They respond to specific factor ligands by altering gene expression in a cell-, developmental-, and sex-specific manner. Three subtypes of PPAR are expressed in different tissues, PPARα, PPARβ (also called PPARδ and NUC1), and PPARγ. Each receptor has the possibility of responding to different ligands, altering the expression of different target genes, and playing different biologic roles (Vanden Heuvel 1999a).

The biologic effects of PPARs are mediated by altered gene expression. Peroxisome proliferator response elements, consisting of imperfect direct repeats of the sequence TGACCT spaced by a single base pair, have been identified in the upstream regulatory sequences of several PPAR target genes. The ligand 9-*cis*-retinoic acid enhances PPAR action by activating the retinoid X receptor, which forms a heterodimer with PPAR and binds to the peroxisome proliferator response elements to induce gene transcription.

Of the three PPAR subtypes, PPARα is the key receptor for peroxisome proliferators. A prototypical marker of peroxisome proliferator action is induction of the peroxisomal enzyme acyl-CoA oxidase, which is elevated about 10-fold in the livers of treated rodents. Additional peroxisome-proliferator-responsive genes include other peroxisomal beta-oxidation enzymes and members of the cytochrome P-450 IVA family. PPARα activation mediates pleiotropic effects such as stimulation of lipid oxidation, alteration in lipoprotein metabolism, and inhibition of vascular inflammation. PPARα activators increase hepatic uptake and esterification of free fatty acids by stimulating the fatty acid transport protein and acyl-CoA synthetase expression. The

carcinogenic effects of PPARα ligands are thought to be associated with altered cell proliferation resulting from the regulation of growth regulatory genes (IAS 2005).

Since the mid-1970s, it has been demonstrated that peroxisome proliferators induce hepatomegaly and hepatocarcinogenicity in rodents (Moody and Reddy 1978; Reddy and Lalwai 1983; Klaunig et al. 2003). Although it has been hypothesized that peroxisome proliferation is causally linked to peroxisome proliferator-induced liver cancer, the direct link between peroxisome proliferation and liver cancer is uncertain. As described below, the two conclusively demonstrated causally linked mechanistic changes of PPARα agonist-induced hepatocellular carcinogenesis in rodents are increased activation of PPARα and PPARα-dependent hepatocyte proliferation. It is well accepted that these agents do not act by a genotoxic (DNA reactive) process. The role of PPARα in the hepatocarcinogenicity of peroxisome proliferators is clearly demonstrated by the fact that these chemicals do not induce hepatomegaly and hepatocarcinogenicity in PPARα null mice (Klaunig et al. 2003). In rodents, peroxisome proliferators lead to tumors that are histologically adenomas or carcinomas that are characterized as basophilic and have absence of γ-glutamyl transpeptidase expression (Kraupp-Grasl et al. 1990; Grasl-Kraupp et al. 1993). These findings suggest that the development of liver tumors by peroxisome proliferators involves amplification of a specific subtype of altered hepatic foci. Some studies have suggested that peroxisome proliferators and PPARα activation might result in tumors in extrahepatic organs. For example, certain peroxisome proliferators increase Leydig cell and pancreatic ademomas and carcinomas (Klaunig et al. 2003). However, whether these are PPARα dependent is unknown.

Marked species differences are observed in response to peroxisome proliferators in terms of peroxisome proliferation and hepatocarcinogenesis. Rats and mice are the most sensitive, and hamsters show an intermediate response, whereas guinea pigs, monkeys, and humans appear to be relatively insensitive or nonresponsive at doses that produce a marked response in rodents (Lai 2004). Nonhuman primates and humans appear to be resistant to the induction of peroxisome proliferation and the development of liver cancer by PPARα agonist drugs (fibrates). To examine the mechanism determining species differences in peroxisome proliferator response between mice and humans, a PPARα-humanized mouse line was generated. The PPARα-humanized and wild-type mice responded to treatment with peroxisome proliferators, as shown by the induction of genes encoding peroxisomal and mitochondrial fatty-acid-metabolizing enzymes and a resultant decrease of serum triglyceride concentrations. However, only the wild-type mice (and not the PPARα-humanized mice) exhibited hepatocellular proliferation (IAS 2005).

Thus far, epidemiologic studies on peroxisome proliferators have shown little evidence of carcinogenic effects in humans (Lai 2004).

APPLICABILITY TO HUMAN HEALTH RISK ASSESSMENT

There is considerable debate about the mechanisms by which peroxisome proliferators cause liver tumors in rodent models and whether these chemicals represent a human cancer risk. It has been well-established that human liver and hepatocytes in culture are less sensitive to the peroxisome proliferation effects of these chemicals; however, as stated above, this event is only associative and not causal to the development of tumors.

Two clinical trials also have examined relative mortality and cancer rates in human males treated with fibrates. In the Helsinki Heart Study, 4,081 men aged 40-55 years with elevated serum cholesterol were treated with either gemfibrozil or placebo for 5 years (Frick et al 1987; Huttunen et al. 1994). Despite significant lowering of serum lipids, which prevented coronary heart disease in the gemfibrozil-treated group, no differences in total death rate or liver cancer incidence were observed between the groups. Liver cancer incidence was not reported as a separate end point; the incidence was reported either as total deaths from cancer or as deaths from liver, gallbladder, and intestinal cancers combined. Except for a borderline statistically significant difference ($P = 0.062$) in the incidence of basal cell carcinomas of the skin between 2,051 patients treated with gemfibrozil and 2,030 subjects receiving a placebo, no differences were found for other cancers. The incidence of cancer mortality in this study, for placebo and fibrate-treated patients, was less than 2% for each group, compared with virtually 100% in PPARα agonist-treated rodents (see above).

The other randomized clinical trial was conducted by the World Health Organization to determine whether clofibrate would lower the incidence of ischemic heart disease in men. A group of 15,745 men were treated with clofibrate and two control groups (one with high cholesterol and one with low cholesterol) of about 5,000 men each were followed for an average of 5.3 years. Follow-up reports were provided 4.3 and 7.9 years after this period. Clofibrate was reported to cause a statistically significantly higher age-adjusted total mortality compared with the high cholesterol placebo-treated control groups in this study. The excess mortality was due to a 25% increase in noncardiovascular causes—that is, diseases of the liver, gallbladder, pancreas, and intestines, including malignant neoplasms of these sites (Committee of Principal Investigators 1980). However, in the final follow-up study (5.3 years in the treatment phase with 7.9 years follow-up for a total of 13.2 years), neither the difference in the number nor the difference in the rate of cancer deaths between the clofibrate-treated group and the control groups was statistically significant (Committee of Principal Investigators 1984). The reason for the difference in mortality at the earlier time point is uncertain. Similar to the Helsinki Heart Study, no data on the incidence of liver cancer alone were provided. In this final follow-up study, there was a 12% excess of deaths from all causes other than ischemic heart disease compared with 25% in the previous studies. Furthermore, the proportional difference between the treated group and the control groups in the final follow-up study was diminished for malignant disease but increased for nonmalignant diseases. The results indicate that the excess in deaths from diseases other than ischemic heart disease was largely confined to the clofibrate-treatment period (average 5.3 years). However, 7.9 years posttreatment, there were 27 deaths associated with liver, gallbladder, and intestinal cancers in the clofibrate-treated group, compared with 18 and 11 deaths associated with the same end points in the high-cholesterol and low-cholesterol control groups, respectively. Similar to the Helsinki Heart Study, this incidence is less than 1% per group.

Data concerning the human susceptibility to liver cancer from peroxisome proliferators come primarily from species comparisons of short-term responses, such as proliferation of peroxisomes in liver parenchymal cells, hepatomegaly, and the induction of various hepatic enzymes and of PPARα expression. Given the importance of PPARα in mediating the short- and long-term effects of PPARα agonist exposure in mice and rats, including liver cancer, cancer risk in humans has been gauged in part by comparing the properties of PPARα among susceptible rodent species and humans. Transient transfection studies using a human PPARα cDNA show that this receptor can transactivate reporter constructs, providing indirect evidence that the

human PPARα is functional. Thus, it is not surprising that peroxisome proliferator response elements have been described in human genes that are transcriptionally regulated by PPARα, including human apo C-III, lipoprotein lipase, apo A-I, apo A-II, carnitine palmitoyltransferase-I, and acyl-CoA oxidase. Interestingly, large increases in the expression of marker mRNAs and proteins, including peroxisomal acyl-CoA oxidase, are not found in human and nonhuman primate hepatocytes treated with these chemicals in vitro. These observations are consistent with a recent study demonstrating the lack of an increase in acyl-CoA oxidase mRNA in human liver samples from 48 patients treated with one of several fibrates (bezafibrate, fenofibrate, or gemfibrozil), despite significant induction of hepatic apolipoprotein A-I mRNA and lowering of serum lipids after treatment. However, dose-dependent induction (<3-fold) of acyl-CoA oxidase activity has been observed in human hepatocytes treated with clofibrate and ciprofibrate, and treatment with perfluorodecanoic acid resulted in significant induction of peroxisomal density and increased acyl-CoA oxidase activity in human cells derived from glioblastoma. Human PPARα activation is reported to result in increased apolipoprotein A-II and lipoprotein lipase transcription and reduced apolipoprotein C-III, which is key to lowering serum triglycerides.

Although data are available indicating that cultured human hepatocytes do not exhibit increased markers of cell proliferation in response to exposure to PPARα agonists while cultured rodent hepatocytes do, this model system might be inappropriate for evaluating this effect because primary hepatocytes do not proliferate substantially in vitro compared with hepatocytes in vivo. Combined, the preceding observations demonstrate that there is some overlap in target gene activation between humans and rodents and that further characterization is required to determine the reasons for the differences. Several possible explanations have been postulated to account for this disparity. There are dramatic differences in the expression levels or function of the expressed protein. Guinea pig liver also was reported to contain significantly less PPARα than mouse liver. However, given the relatively small number of human liver samples examined, further quantification of PPARα mRNA and protein in addition to other transcription factors to serve as good positive controls would be important. More recently, data from the humanized PPARα mouse suggest that inherent differences in receptor activation might contribute to the observed species difference in hepatocarcinogenicity, rather than receptor expression level.

It appears that lower levels of PPARα expression might, in part, contribute to the species differences between rodents and humans, but expression of truncated or mutant PPARα, some through expression of alternatively spliced products, also has been described. No mutations or polymorphisms have been described to date in rodent species. A dominant-negative form of human PPARα has been described, and the presence of this protein could significantly inhibit PPARα activation and subsequent target gene modulation. Additionally, separate laboratories have described two different mutations (L162V and V227A) in human PPARα. The biologic significance of these mutant human isoforms of PPARα is unclear but has been linked to significant differences in serum apolipoproteins, serum cholesterol, and fibrate-induced changes in serum high density lipoprotein cholesterol. Some of the mutant PPARα proteins were shown to act as dominant-negative proteins in that they could prevent the normal PPARα from interacting with retinoid X receptor, binding to the peroxisome proliferator response element, and activating gene expression. These findings suggest that, in addition to reported lower expression of human PPARα, mutant variants, in particular a dominant-negative isoform, also might contribute to the apparent insensitivity in humans to PPARα agonists. However, because

some humans respond to fibrate therapy, the hypothesis that altered PPARα protein accounts for the species differences is likely not true for all human cell types that express PPARα.

The available epidemiologic and clinical studies are inconclusive but, nonetheless, do not provide strong evidence that PPARα agonists cause liver cancer in humans. Evidence from an in vivo model suggests that there could be considerably more similarities in PPARα target genes among humans and rodents. The weight of evidence suggests that this mode of action would be plausible in humans because they possess PPARα in sufficient quantities to mediate the human hypolipidemic response to therapeutic fibrate drugs. Thus, human PPARα is comparable to rat or mouse PPARα in its affinity for some, but not all, PPARα ligands, and evidence from the humanized PPARα mouse suggests that there are differences in target genes activated between human and rodent PPARα. However, a point in the rat and mouse key events cascade where the pathway is biologically precluded in humans in principle cannot be identified. Whereas the mode of action is plausible in humans, the weight of evidence suggests that this mode of action is not likely to occur in humans based on differences in several key steps when taking into consideration kinetic and dynamic factors. There is no convincing evidence for human tumorigenicity resulting from exposure to the PPARα agonists di-(2-ethylhexyl)phthalate and various hypolipidemic fibrates. The studies of di-(2-ethylhexyl)phthalate are not considered adequate to demonstrate lack of tumor hazard but do constitute some evidence of absence. In contrast, there are extensive data for some of the hypolipidemic drugs, particularly clofibrate. The International Agency for Research on Cancer concluded that "the mechanism of liver carcinogenesis in clofibrate treated rats would not be operative in humans" based on the results of extensive epidemiologic studies (IARC 1996), particularly the World Health Organization trial on clofibrate comprising 208,000 man-years of observation (Committee of Principal Investigators 1980, 1984). Further, a meta-analysis of the results from six clinical trials on clofibrate found no excess cancer mortality. These human data are supported by evidence from nonhuman primate studies that show no evidence of tumors or focal lesions over 6-7 years of exposure. However, it is important to point out that many of the molecular and biochemical changes just described have not been evaluated in nonhuman primates with recently developed, high-affinity PPARα agonists. Most of the work to date has been performed and published using PPARα agonists with relatively low (di-[2-ethylhexyl]phthalate) or moderate (fibrate class of hypolipidemic drugs) affinity for activation of PPARα.