United States
Environmental Protection
Agency

Office of Research and
Development
Washington DC 20460

EPA/600/6-91/002A
February 1991
SAB Review Draft

EPA

Response to Issues and Data Submissions on the Carcinogenicity of Tetrachloroethylene (Perchloroethylene)

Review Draft
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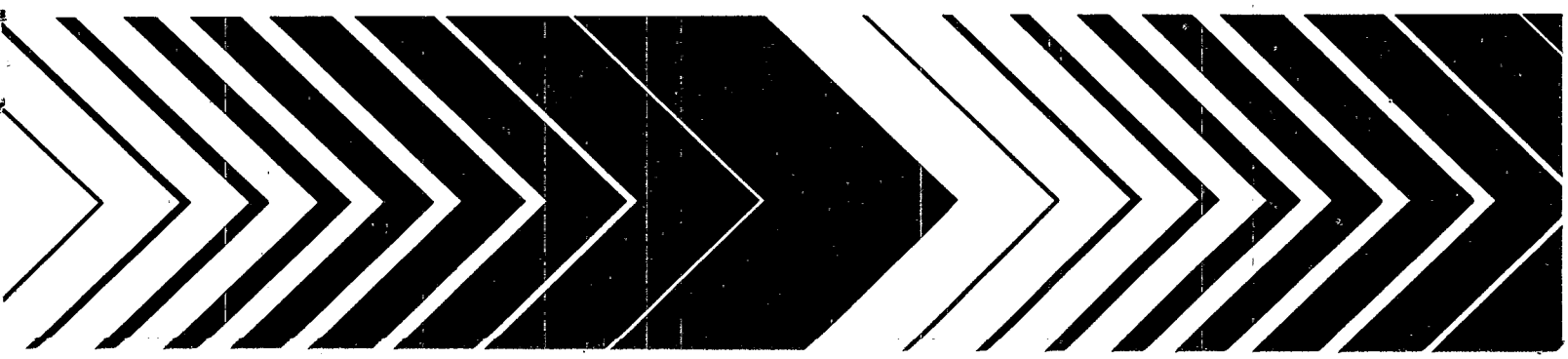
This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 22 1991

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Charge to Science Advisory Board Regarding Review of "Response to Issues and Data Submissions on the Carcinogenicity of Tetrachloroethylene"

FROM: William H. Farland, Ph.D.
Director
Office of Health and Environmental Assessment (RD-689)

TO: Donald G. Barnes
Executive Director
Science Advisory Board (A-101)

The Office of Health and Environmental Assessment (OHEA) appreciates the Board's agreement to meet on March 26, 1991 to review the above-titled document.

By way of background, the subject of a potential cancer hazard from tetrachloroethylene (perchloroethylene-PCE) is not a new one in terms of past SAB/EPA dialogue. The most recent correspondence was a letter of advice dated March 9, 1988 from the Board to the Administrator regarding the Board's perspectives on this topic. Since 1988, OHEA has been monitoring research findings relevant to PCE's carcinogenic potential.

This "response" document has a relatively narrow purpose, as stated in the introduction, compared to the more typical comprehensive health assessment document that the committee usually reviews for OHEA. The objective is to revisit issues and review data concerning the identification of hazard, i.e., the weight of the animal evidence bearing on the potential for human carcinogenicity. Data have been submitted and issues raised in public comment connected with a variety of recent Agency rule-making actions. We have also updated our own literature collection on relevant topics. An earlier version of this response document is currently in the docket for the recently promulgated National Primary Drinking Water Standard for Tetrachloroethylene as published in the Federal Register on January 30, 1991.

In general terms, we request that the Board review the technical adequacy of discussions concerning the animal cancer data and related ancillary information, such as mutagenicity and metabolism, and the relationship of this information base to a hazard classification of PCE under the Agency's current cancer guidelines. More specifically, we request that the Board's focus include the topics or questions listed below.

(1) Technical adequacy of discussions about the three animal bioassay tumor endpoints, particularly regarding the relevance of these tumor endpoints to the potential for human hazard at some dose.

(2) Technical adequacy of discussions about ancillary information for mutagenicity and metabolism considerations and the appropriate use of this information in providing a better understanding of the animal bioassays or the relevance of these to the potential for human hazard.

(3) Have all important issues been identified and appropriately considered, recognizing that many more fundamental scientific questions may exist but which may not be developed adequately to meaningfully discuss in a risk assessment context?

(4) The soundness of the rationale used to weigh the evidence, from each endpoint and in the aggregate for human hazard potential. This topic relates to the logic of weighing animal evidence including the relevance of ancillary data to that process.

It is important to note that the concept of "weight of evidence" under EPA's Cancer Risk Assessment Guideline identifies an agent's potential to be a human hazard at some dose. Questions about the quantitative relationship of dose to response and mechanisms of action that affect that relationship are dealt with as a separate quantitative dose-response assessment which typically follows the hazard identification part of a comprehensive assessment. The quantitative dose-response relationships for tetrachloroethylene needs to be revisited, as noted in the response document, and we will be doing so in the future.

We look forward to the Board's advice on this health hazard identification topic. Should you decide that an overview of carcinogen risk assessment guideline criteria for weighing the evidence is useful for background purposes, we will make agenda arrangements with Sam Rondberg.

cc: Erich Bretthauer

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EPA/600/6-91/002A
February 1991
SAB Review Draft

RESPONSE TO ISSUES AND DATA SUBMISSIONS ON THE CARCINOGENICITY OF TETRACHLOROETHYLENE (PERCHLOROETHYLENE)

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

Human Health Assessment Group
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CONTENTS

PREFACE

This document was prepared by the Office of Health and Environmental Assessment (OHEA) to respond to data and comments submitted to the Agency and to discuss how this information influences the overall weight-of-evidence classification for a perchloloroethylene human cancer hazard. Relevant literature through the fall of 1990 has been critically evaluated.

AUTHORS

Members of the Human Health Assessment Group of the Office of Health and Environmental Assessment (OHEA) prepared this document.

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1. INTRODUCTION

The scientific debate over the potential carcinogenicity of tetrachloroethylene (perchloroethylene, perc, PCE) spans several years. The Office of Health and Environmental Assessment within the U.S. Environmental Protection Agency's Office of Research and Development has been considering the issues and current thinking pertaining to weight of evidence for the human cancer hazard from exposure to perchloroethylene. Several issues were brought up by the EPA's Science Advisory Board (SAB, 1987, 1988) during their review of an addendum (U.S. EPA, 1986a) to the Health Assessment Document for Tetrachloroethylene (U.S. EPA, 1985). New information also has become available over the last two to three years that has bearing on the issues.

Recently generated laboratory data have led to the development of hypotheses about the mechanisms of perchloroethylene tumorigenesis. Biological arguments have been put forward suggesting species specificity for some of the proposed tumorigenic mechanisms. Such arguments imply that certain experimental results are of questionable predictive validity with respect to human health hazards and risks. While there is some evidence to support these arguments, several critical experimental elements are missing to determine cause and effect relationships in the test animals or to answer the human relevancy question with certainty. Since the data are equivocal regarding mechanisms, a conclusion about the carcinogenic potential of perchloroethylene in humans must be one of judgment considering the weight of the pertinent evidence.

The objectives of this paper are: to review the current issues and hypotheses surrounding perchloroethylene carcinogenesis, to evaluate these hypotheses in light of recently published studies, and to develop the EPA's response to issues and data submitted in comments to the Agency on the overall weight of evidence for potential human cancer hazard. This paper reviews the issues considered by the EPA's Science Advisory Board during its review of the draft "Addendum to the Health Assessment Document for Tetrachloroethylene" (U.S. EPA, 1986a) and discusses relevant research data that have been published since 1986. The topics covered include three tumor end points observed in rodents exposed to perchloroethylene:

1. hepatocellular carcinoma in male and female mice,
2. renal tubule neoplasia in male rats, and
3. mononuclear cell leukemia in male and female rats,

and the recent data on metabolism, genotoxicity and mutagenicity, peroxisome proliferation, and alpha-2μ-globulin.

The EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986b) provide a framework for assessing the likelihood of a substance being a human cancer hazard. Under these guidelines animal studies and human data are first analyzed separately. The evidence from laboratory animal studies, along with other relevant information, may be classified as "sufficient," "limited," "inadequate," "no data," or "no evidence" in animals. The human data is classified as "sufficient," "limited," "inadequate," "no data," or "no evidence" in humans. The two sets of information are merged with respect to assessing potential carcinogenicity in humans (see Appendix A: Guidelines for Carcinogen Risk Assessment, 1986). The classifications refer only to the weight of the experimental evidence that a chemical is carcinogenic and not to its potency of carcinogenic action. The overall challenge here is not only to determine how the currently available data influence the Agency's previous categorization of the experimental animal evidence on the carcinogenicity of perchloroethylene as "sufficient," but also to determine whether the "sufficient" animal data in the case of perchloroethylene signify a human hazard potential as would be ordinarily assumed (U.S. EPA, 1986b; OSTP, 1985; IARC, 1982, 1987).

2. BACKGROUND

2.1. PRIOR EPA ANALYSES

The EPA published a Health Assessment Document (HAD) for Tetrachloroethylene (Perchloroethylene) in July of 1985 (U.S.EPA, 1985). The Office of Health and Environmental Assessment (OHEA), in consultation with an Agency workgroup, prepared the HAD to serve as a source document for the entire EPA (U.S.EPA, 1985, preface). The document underwent extensive expert peer review and review by the Environmental Health Committee of the Agency's Science Advisory Board (SAB) prior to publication. Based on the EPA's interpretation of the overall weight of evidence, the HAD placed perchloroethylene into Group C (possible human carcinogen). This categorization was in accordance with the Agency's proposed Guidelines for Carcinogen Risk Assessment (published in final form in September, 1986). The classification was based primarily on the finding that "in a gavage bioassay, perchloroethylene induced a statistically significant increase of malignant liver tumors in both male and female B6C3F1 mice." This decision reflected a "limited" number of studies showing a robust positive response, plus the fact that the response was a commonly observed tumor type as opposed to a very rare tumor type, rather than "limited" evidence, such as a borderline response, derived from a number of adequately run studies. In view of the pending release of National Toxicology Program (NTP) reports on long-term animal inhalation studies with perchloroethylene, the HAD stated that the carcinogenicity conclusions were interim, however, and would be updated if necessary when the NTP reports were evaluated (U.S.EPA, 1985, preface).

A draft "Addendum to the HAD for Tetrachloroethylene (Perchloroethylene)" (U.S. EPA, 1986a), prepared by OHEA, analyzed the results of the inhalation bioassays conducted by the NTP and performed by Battelle Pacific Northwest Laboratories. The results of these studies revealed perchloroethylene-associated increases in the incidences of hepatocellular carcinomas--the same tumor type seen in the gavage study--in both sexes of B6C3F1 mice, mononuclear cell leukemia in both sexes of F 344/N rats, and uncommon renal tubule neoplasms and some evidence for gliomas of the brain in male rats. The authors of the addendum concluded that perchloroethylene is a B2 chemical (probable human carcinogen) because:

- the NTP inhalation bioassay demonstrated that perchloroethylene can induce carcinogenic effects at multiple sites, in both rats and mice (a replicate finding) through inhalation exposure (a second route), and
- the earlier NCI bioassay provided positive evidence of hepatocellular carcinomas in mice administered perchloroethylene by gavage.

On May 15, 1986, the draft addendum received peer review by the Halogenated Organics Subcommittee of the EPA's SAB in a public meeting held in Madison, Wisconsin. The SAB's initial comments appear in a letter to EPA Administrator Lee Thomas dated January 27, 1987 (SAB, 1987). In this letter the SAB concluded "that perchloroethylene belongs in the overall weight-of-evidence category C (possible human carcinogen)."

Further, the SAB judged the evidence for carcinogenicity in animals to be "limited" because, "the National Toxicology Program bioassay does not provide a scientific basis to associate either lesion (in rats) with inhalational exposure to perchloroethylene," thus, "the evidence arises only from a single strain of mouse" and "the kind of tumor associated with perchloroethylene exposure in this mouse strain makes it difficult to create an inference regarding human carcinogenicity."

As a result of the SAB's conclusions, Agency scientists and managers reexamined the assessment of perchloroethylene and again concluded that perchloroethylene should be classified as a B2 chemical. Administrator Thomas responded to the SAB in an August 3, 1987, letter that clarified the Agency's position on perchloroethylene, and additional consultative advice was requested of the SAB on specific issues regarding liver tumors in B6C3F1 mice and kidney tumors in male rats. More detailed comments were presented in "EPA Staff Comments on Issues Regarding the Carcinogenicity of Perchloroethylene (Perc) Raised by the SAB," a paper that was enclosed with the Administrator's August 3 letter.

The SAB's response to the second request for advice is contained in a letter dated March 9, 1988, to Administrator Thomas (SAB, 1988). In this letter the SAB concluded that, "the overall weight of evidence lies on the continuum between the categories B2 and C of EPA's risk assessment guidelines for cancer." In an attempt to put this conclusion in perspective, the SAB also remarked that:

> A substance classified as C (limited evidence in animals) for which human exposure is high may represent a much greater potential threat to human health. EPA and other agencies (including those in state governments) may, therefore wish to take steps to reduce high exposures to substances in the C category whenever there appears to be a potentially significant threat to human health (in the sense that the plausible upper bound estimate of potency times lifetime exposure is above the threshold where regulation may be judged appropriate).

Since that time, the EPA has received public comments on perchloroethylene issues in several regulatory actions which include the perchloroethylene weight-of-evidence classification as a matter considered. These public comments pertain to RCRA listing (Federal Register, Dec., 1989) and CERCLA reportable quantity rules (Federal Register, August, 1989), and to MCLG and MCL proposals for drinking water (Federal Register, May, 1989; December, 1990).

2.2. ANIMAL STUDIES OF PERCHLOROETHYLENE CARCINOGENICITY

Perchloroethylene has shown cancer-causing activity in male and female mice and in male and female rats in the NCI/NTP studies. In both sexes of mice, perchloroethylene induced dose-related statistically significant increases in hepatocellular carcinomas when administered by oral gavage or by inhalation. Statistically significant increased incidences of mononuclear cell leukemia, and the presence of uncommon renal tubule neoplasms and some evidence of gliomas in the brain were observed in male rats exposed to perchloroethylene by inhalation. The renal tubule tumors were also detected in male rats exposed by gavage. Female rats exhibited an increase of mononuclear cell leukemia when exposed to perchloroethylene by inhalation.

Although perchloroethylene increased the incidence of cancer at three different sites and in two species, controversy surrounds each of the tumor end points. Considerable scientific debate has focused on the predictive validity of mouse liver and male rat kidney tumors as well as mononuclear cell leukemia. In addition to the general controversies surrounding these tumor end points, chemical-specific data that may be pertinent to the evaluation of the effect of perchloroethylene on tumor incidence has generated concern.

2.3. PURPOSE OF THIS PAPER

The intent of this paper is to respond to data and comments submitted to the Agency. Studies related to perchloroethylene tumorigenesis have been published subsequent to EPA's 1986 draft Addendum, and have been formally submitted to the EPA. These studies were designed to elucidate the mechanism of action in animals and provide better understanding of the relevance of animal data to human hazard. The studies report mechanistic data germane to a number of issues concerning the etiology of perchloroethylene-induced rodent tumors and their relevance to humans. The EPA must evaluate information from these studies and report its assessment of whether the data provide an understanding of underlying tumorigenesis mechanisms that would lead to a conclusion that modes of perchloroethylene cancer-causing activity are not operative in humans. The purpose of this paper then, is to provide a response to the data and comments submitted to the Agency that have bearing on the relevancy issues, and to discuss how this information influences the overall weight-of-evidence classification for a perchloroethylene human cancer hazard.

This paper summarizes OHEA's critical evaluation of three perchloroethylene tumor end points in rats and mice in light of new scientific findings, and incorporates the recent information into the weight of evidence for human hazard.

The paper addresses recent literature on perchloroethylene and its biometabolites as the information relates to: metabolism, mutagenicity, cytotoxicity and proliferative changes in mouse liver, nephrotoxicity and renal tubule neoplasia in male rats, and mononuclear cell leukemia in male and female rats.

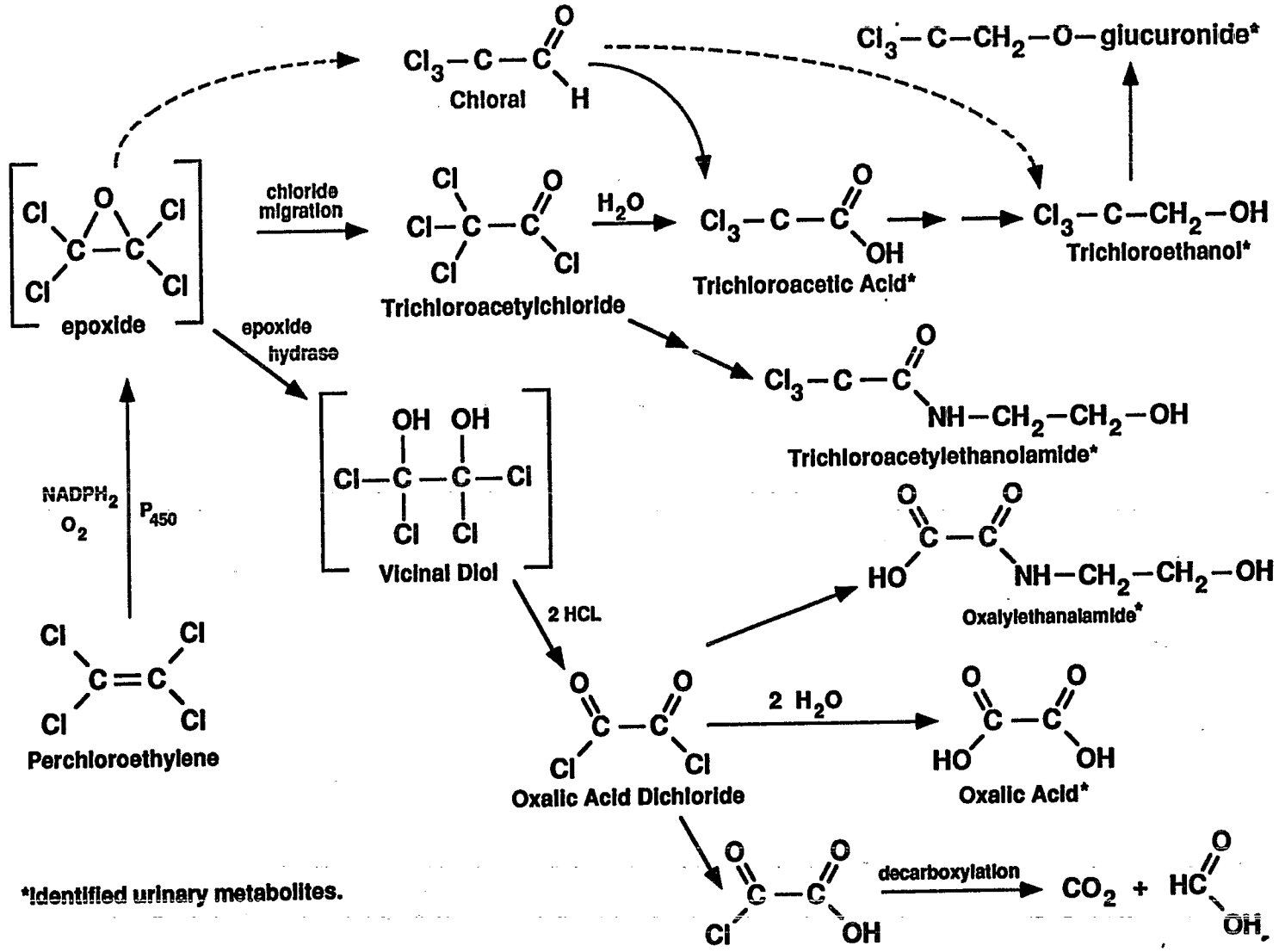
3. THE METABOLISM OF PERCHLOROETHYLENE

The cancer-causing activity of halogenated ethylenes is generally considered to reside primarily in biometabolites rather than in the parent compounds themselves. Studies in both animals and humans indicate that metabolism of perchloroethylene is relatively limited, as evidenced by the fact that a high percent of absorbed dose is excreted in the breath as the parent molecule. In human studies, however, only approximately half of the perchloroethylene absorbed has been accounted for through the excretion of parent compound or metabolites.

Estimates of the extent of metabolism in humans have been made from balance studies by accounting for a retained dose after inhalation exposure by measuring trichloro-compounds excreted in the urine. Metabolites other than those measured may be excreted in the urine or bile. Thus, the additional perchloroethylene in humans may be metabolized to compounds that were not measured. Other as yet unrecognized pathways for perchloroethylene that have not been taken into consideration may exist in humans.

Perchloroethylene is metabolized through at least two distinct pathways. Oxidative metabolism via the cytochrome P_{450} system has been extensively reviewed in the HAD (U.S. EPA, 1985). Recent investigations have revealed a glutathione conjugative pathway which appears to be a minor but important route that has been shown to generate a mutagenic constituent. The oxidative and conjugative pathways are summarized in Figures 1, 2, and 3.

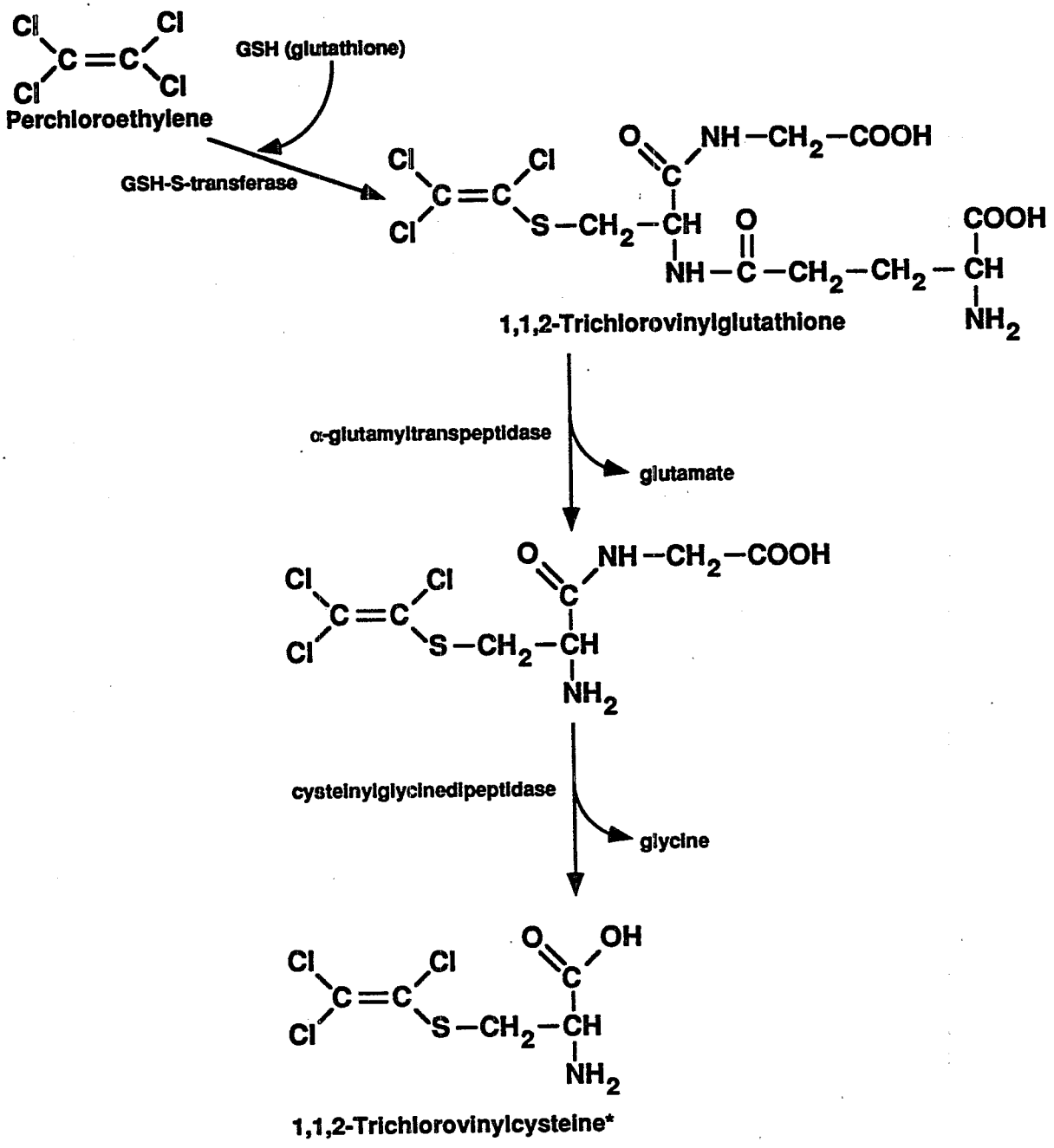
Oxidative metabolism of perchloroethylene (dependent on cytochrome P_{450}) probably occurs mostly in the liver but may occur at other sites. This pathway is operative in humans as well as rodents and leads to the production of several metabolites (Figure 1). There is no basis to believe that qualitative differences exist between species with respect to known pathways of oxidative metabolism of perchloroethylene. However, there are quantitative differences among the metabolic rates of different species. The major metabolite of the oxidative pathway is trichloroacetic acid (TCA) which is excreted in the urine of all species tested. Other identified urinary metabolites are designated in Figure 1. Some of the intermediates in the oxidative pathway are known to possess cytotoxic/genotoxic activity (e.g.,



*Identified urinary metabolites.

Figure 1. Oxidative Metabolism of Perchloroethylene. The oxidative pathway is operative in humans as well as in rodents and leads to the production of several metabolites, some of which are mutagenic and/or carcinogenic.

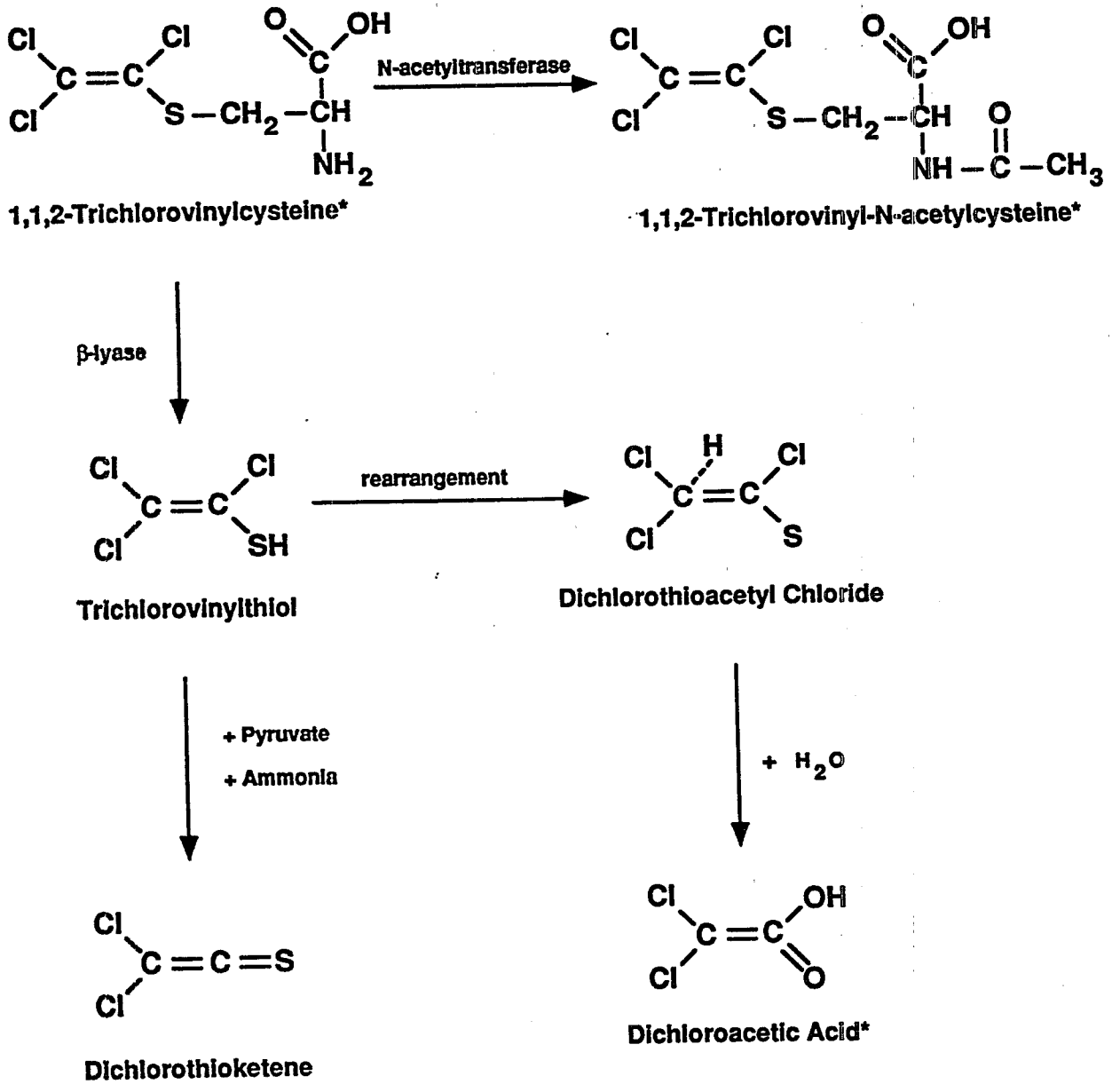
SOURCE: U.S. EPA, 1985, 1985b.



*Identified urinary metabolites.

Firgure 2. Enzyme-catalyzed metabolism of perchlorothylene to its glutathione conjugate, followed by removal of the glutamyl and glycine residues to yield its corresponding cysteine S-conjugate. Conjugation of perchloroethylene with glutathione has been demonstrated in rat and mouse hepatic cytosolic and microsomal fractions. Further processing to 1,1,2-trichlorovinylcysteine occurs in the kidney. This metabolite gives rise to potent mutagens.

SOURCE: Anders *et.al.*, 1988.



*Identified urinary metabolites.

Figure 3. Further metabolism in the kidney of the perchloroethylene intermediate 1,1,2-trichlorovinylcysteine, leading to mutagenic metabolites. These pathways occur in humans as well as in rodents.

SOURCE: Dekant *et.al.*, 1987; Green, 1990; ECETOC, 1990.

perchloroethylene-epoxide, chloroacetaldehydes; see Section 4) and several have been shown to cause cancer (e.g., DCA, TCA, and chloroacetaldehydes).

Recent studies in rats (reviewed by Anders et al., 1988 and DeKant et al., 1989) have demonstrated the formation of a cytotoxic and mutagenic metabolite(s) of perchloroethylene that arises from hepatic glutathione conjugative pathways (see Figures 2 and 3). This secondary metabolic pathway is initially catalyzed by hepatic cytosolic and microsomal glutathione S-transferases to yield S-(1,2,2-trichlorovinyl) glutathione (TCVG). After transport to the kidney, TCVG is metabolized to S-(1,2,2-trichlorovinyl) cysteine (TCVC) by the enzymatic removal of glutamyl and glycine residues. TCVC is acetylated to N-Acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine, which is excreted in the urine. However, TCVC is also a substrate for renal beta-lyases which cleave TCVC to yield an unstable thiol that may give rise to cytotoxic and mutagenic intermediates.

In vivo and in vitro experiments in rodents provide evidence to support this conjugative metabolic scheme. DeKant et al. (1987) and Green et al. (1990) have demonstrated hepatic conjugation of perchloroethylene with glutathione by rat liver fractions in in vitro experiments. Vamvakas et al. (1989) found TCVG in the bile excreted by perchloroethylene-perfused rat livers, and Green et al. (1990) reported the presence of the glutathione conjugate in the bile of rats administered perchloroethylene by gavage.

There is some limited evidence suggesting that humans may not metabolize perchloroethylene by the conjugative pathway. Human liver samples have been compared to rat and mouse liver samples with respect to ability to conjugate perchloroethylene with glutathione (Green et al., 1990). These investigators found low levels of conjugation by rat and mouse livers but were unable to demonstrate conjugation by human livers. To confirm the viability of glutathione S-transferase in the rat and human liver samples studied using a more powerful protocol, these workers compared the two species with respect to their ability to conjugate 1-chloro-2,4-dinitrobenzene with glutathione. Both species carried out this conjugation rapidly and at essentially the same rate. This finding indicates that the reduced ability of the human liver samples to conjugate perchloroethylene was not attributable to an inactive glutathione S-transferase. Because of the very low levels of enzyme activity being measured and the limited numbers of human liver samples tested, it is premature to conclude

that humans are unlikely to carry out this metabolic step. Additional confirmatory studies are clearly needed.

The beta-lyase pathway has been shown to produce cytotoxic and mutagenic metabolites from glutathione and cysteine conjugates of a variety of haloalkenes in a number of animal models in vivo or in vitro (Anders et al., 1988; Lock, 1988). The same pathway which leads to the formation of the toxic metabolites in animal models and mutagenic metabolites in bacterial models is also present in human proximal tubular cells. Human proximal tubular cells have been shown to be sensitive to the toxicity of glutathione and/or cysteine conjugates of a variety of chloro- and fluoroalkenes that are activated via the beta-lyase pathway (Chen et al., 1990).

4. MUTAGENICITY OF PERCHLOROETHYLENE AND ITS METABOLITES

Genetic alterations are critical events in the carcinogenesis process. Thus, evidence on the ability of an agent to produce heritable genetic lesions (e.g., gene mutations, stable chromosomal aberrations, aneuploidy) can potentially provide useful mechanistic information for induced carcinogenesis. Additionally, it is reasonable to assume that mutagenic mechanisms are universal, and thus evidence of mutagenesis is also regarded as important information that supports the inference of potential for carcinogenicity in humans. It should be emphasized, however, that genetic alterations are only one component of carcinogenesis. Moreover, the most commonly used assays in genetic toxicology are in vitro ones. Thus, the observations of mutagenic noncarcinogens and nonmutagenic carcinogens as well as mutagenic carcinogens are to be expected. The use of results from short-term genotoxicity tests as supporting evidence for or against carcinogenicity of an agent must be undertaken with caution.

The 1985 EPA Health Assessment Document for Tetrachloroethylene provided a comprehensive assessment of the genotoxicity of perchloroethylene. The conclusion reached in the document was that the available data did not clearly support a mutagenic potential for perchloroethylene. The evidence available at that time indicated that if perchloroethylene is mutagenic, it is only weakly so. An update of the scientific literature was recently conducted to determine if the earlier conclusions are still valid.

4.1. DATA ON MUTAGENICITY OF PERCHLOROETHYLENE PER SE

As shown in Table 1, perchloroethylene has not been clearly shown to be an inducer of gene mutations in routinely used assays. In bacterial assays for reverse mutation (*Salmonella*/mammalian microsome test) in the presence or absence of exogenous liver S9 activation, perchloroethylene exposures produced largely negative results (Bartsch et al., 1979; Margard, 1978; Haworth et al., 1983; Warner et al., 1988). In studies reporting positive results, the responses were weak and were produced by cytotoxic concentrations. There was no evidence of clear dose-related effects. These positive findings may have also been related to the presence of mutagenic contaminants and/or stabilizers in the

TABLE 1. Summary of Genotoxicity Testing of Tetrachloroethylene

A. Gene Mutation Tests	Results[a]	References
Salmonella/Ames assay	mostly - +[b]	Shimada et al., 1985; Margard, 1978; SRI International, 1983; Bartsch et al., 1979; Haworth et al., 1983; Warner et al., 1988
Escherichia coli K12/343/113 ( ) Multi-purpose test	-	Henschler, 1977; Greim et al., 1975
Yeast reverse mutation test	-	Calen et al., 1980; Bronzetti et al.; 1983
Drosophila sex-linked recessive lethal test	-	Beliles et al., 1980; Valencia et al., 1985
L5178YTK^{+}/TK^{-} mouse lymphoma cell assay	-, ?[c]	Myhr et al., 1986; Galloway et al., 1987; McGregor et al., 1988
B. Chromosomal Aberration Tests		
Chinese hamster ovary cells (CHO)	-	
Rat bone marrow assay	-[c]	Rampy et al., 1978; Beliles et al., 1980
Peripheral lymphocytes from exposed humans	-	Ikeda et al., 1980
C. Other Tests Indicative of DNA Damaging Activity		
Unscheduled DNA synthesis in WI-38	-[c]	Beliles et al., 1980
Hepatocyte primary cluture/DNA repair test	+[b],-	Shimada et al., 1985; Costa and Ivanetich, 1984; Goldsworthy et al., 1988
Mitotic recombination tests in yeast	+,-	Callen et al., 1980; Bronzetti et al., 1983
Sister chromatid exchange formation in CHO cells	-	Koch et al., 1988; Galloway et al., 1987
DNA strand breaks (alkaline elution test assay) in mouse kidney and liver cells	ok	Walles, 1986
D. DNA Binding Studies		
Mice	-	Schumann et al., 1980
Mice and rats	?	Mazzullo et al., 1987

[a]+ designates positive; - negative; wk weak response; ? inconclusive test. Dose-response relationships were not established for the reported + results or wk results.

[b]Positive results are considered weak because large amounts of material were needed to elicit the responses. Results may also be explained by mutagenic stabilizers or contaminants.

[c]Questionable evidence for weak or borderline activity in specific data sets.

perchloroethylene samples tested. When highly purified perchloroethylene was evaluated in a desiccator using the *Salmonella*/mammalian microsome test, negative results were obtained (Shimada et al., 1985)

Perchloroethylene has also tested negative in yeast for reverse mutations (Callen et al., 1980; Bronzetti et al., 1983) and *Drosophila* for sex-linked recessive lethal mutations (Valencia et al., 1985). Responses after perchloroethylene treatment in the L5178Y Tk^{+}/Tk^{-} mouse lymphoma cell assay for forward mutations have been either negative or equivocal (Myhr et al., 1986; McGregor et al., 1988).

Perchloroethylene has not been demonstrated to be a clastogen (chromosome-breaking activity). Negative results were found for the induction of chromosomal aberrations in cultured Chinese hamster ovary cells (Galloway et al., 1987) and in the bone marrow assay for rats and mice (Cerna and Kypenova 1977; Rampy et al., 1978; Beliles et al., 1980). A cytogenetic study of humans exposed to perchloroethylene did not show elevated frequencies of chromosomal aberrations or sister chromatid exchanges in peripheral lymphocytes (Ikeda et al., 1980).

Chemical adduct formation is a prerequisite step in certain types of mutagenesis. Schumann et al. (1980) reported no detectable DNA binding in livers of mice exposed to inhaled ^{14}C - labeled perchloroethylene. This was not a sensitive test, however, because the specific activity of the label was too low to preclude the possibility of DNA binding (i.e., this test could not detect slightly fewer than 10^{-5} alkylations per nucleotide; recent protocols are able to detect 10^{-9} to 10^{-12}). In a more recent study, Mazzullo et al. (1987) reported low levels of DNA binding in the liver, kidney, lung, and stomach of the mouse and rat after intraperitoneal injection of perchloroethylene. These low levels of DNA binding cannot be distinguished from binding as a result of biosynthetic incorporation of the label into DNA, and thus, it is questionable whether exposure to perchloroethylene results in the formation of DNA adducts.

Perchloroethylene exposures have produced negative, questionable, or weak results in tests that do not measure mutation per se but are indicative of DNA damaging activity. Tests for DNA repair synthesis in hepatocytes (Shimada et al., 1985; Costa and Ivanetich, 1984; Goldsworthy et al., 1988), mitotic recombination in yeast (Bronzetti et al., 1983; Koch et al.,

1988), and sister chromatid exchange formation in culture Chinese hamster cells (Galloway et al., 1987) have been predominantly negative. Perchloroethylene has been reported to be a weak inducer of DNA single strand breaks in mouse liver and kidney (Walles, 1986). Although DNA strand breaks are events that may lead to mutagenicity, agents that can be demonstrated to induce only DNA strand breakage should not be viewed to possess the same genetic hazard potential as agents that have been shown to induce gene mutations or stable chromosomal aberrations.

4.2. MUTAGENICITY OF PERCHLOROETHYLENE METABOLITES

At the time the 1985 HAD was being prepared, only a limited literature existed on the mutagenicity of perchloroethylene metabolites. However, several studies are now available (summarized in Table 2) and their results warrant some consideration.

Oxidative metabolism of perchloroethylene (dependent on cytochrome P_{450}) occurs mostly in the liver. This pathway is operative in both rodents and humans and leads to the production of several metabolites (Figure 1). Perchloroethylene-epoxide, a hypothesized intermediate in perchloroethylene oxidative metabolism, has been shown to be mutagenic in the *Salmonella*/mammalian microsome test (Kline et al., 1982).

Chloral hydrate (trichloroacetaldehyde), a known metabolite of trichloroethylene and likely a perchloroethylene metabolite, has been produced under both in vitro and in vivo conditions. Several studies are available on the ability of chloral hydrate to produce aneuploidy (i.e., loss or gain of whole chromosomes) in both mitotic and meiotic cells, including yeast (Singh and Sinha, 1976, 1979; Kafer, 1985; Gualandi, 1987; Sora and Carbone, 1987), cultured mammalian somatic cells (Degrassi and Tanzarella, 1988), and spermatocytes of mice (Russo et al., 1984; Liang and Pacchierotti, 1988). It should be pointed out that this type of genetic effect is most likely due to interference of spindle function rather than a DNA-reactive mechanism. Chloral hydrate has been reported to be weakly mutagenic in the *Salmonella*/mammalian microsome test (Haworth et al., 1983) but negative for sex-linked recessive lethal mutations in Drosophila (Yoon et al., 1985). It has been reported to induce single-strand breaks in hepatic DNA of mice and rats (Nelson and Bull, 1988) and mitotic gene conversion in yeast (Bronzetti et al., 1984). Chloral hydrate also has

TABLE 2. Summary of Genotoxicity Testing of Tetrachloroethylene Metabolites

Metabolite	(Result)*/Assay	Reference
Perchloroethylene-epoxide	(+) Salmonella/Ames Assay	Kline et al., 1982
Chloral hydrate	(+) Aneuploidy/yeast	Singh and Sinha, 1976, 1979; Kafer, 1985; Gualandi, 1987; Sora and Carbane, 1987
	(+) Aneuploidy/mammalian cells in vitro	Degrassi and Tanzavella, 1988
	(+) Aneuploidy/spermatocytes of mice	Russo et al., 1984; Liang and Pacchierotti, 1988
	(wk) Salmonella/Ames test	Haworth et al., 1983
	(-) Drosophila sex-linked recessive lethal mutation	
	(+) DNA strand breaks in mice and rats	Nelson and Bull, 1988
	(+) mitotic gene conversion in yeast	Bronzetti et al., 1984
Trichloroacetic Acid		
	(+) DNA strand break in mice and rats	Nelson and Bull, 1988
	(-) DNA strand break in mice and rats	Chang et al., 1989
	(?) in vivo cytogenetics	Bhunya and Behera, 1987
	(-) Salmonella/Ames Assay	Waskell, 1978
Dichloroacetaldehyde		
	(+) Salmonella/Ames Assay	Bignami et al., 1980
	(+) DNA strand breaks in human cells in vitro	Chang et al., 1989
Monochloroacetaldehyde		
	(+) DNA strand breaks in human cells in vitro	Chang et al., 1989
S-(1,2,2-trichloro-vinyl)gluthione		
	(+) Salmonella/Ames Assay	Vamvakas et al., 1989

*+ designates positive; - negative; wk weak response; ? inconclusive test.

been observed to cause tumors in mice (Rijhsinghani et al., 1986; Daniel, 1990). Other chloroacetaldehydes are potentially mutagenic. Dichloroacetaldehyde (DCAA) is mutagenic in the *Salmonella*/mammalian microsome test (Bignami et al., 1980) and both monochloroacetaldehye and, to a lesser extent DCAA, appear to induce DNA single strand breaks in cultured human cells (Chang et al., 1989).

Few genotoxicity studies are available on the carcinogenic perchloroethylene metabolites trichloroacetic acid (TCA) and dichloroacetic acid (DCA). TCA and DCA have been reported to produce single strand breaks in hepatic DNA of mice and rats. This action is independent of peroxisome proliferation and of liver necrosis (Nelson and Bull, 1988; Nelson et al., 1989). The induction of DNA single strand breaks could not be confirmed by other laboratories, however, a different methodology was used (Chang et al., 1989). TCA was reported as positive for the induction of chromosomal aberrations and micronuclei in the bone marrow of mice (Bhunya and Behera, 1987). This finding is questionable, however, because of the low background frequencies reported for chromosomal aberrations and the anomalous dose-response seen for micronuclei formation in normochromatic erythrocytes.

In rats perchloroethylene has been shown to be metabolized to a cytotoxic, mutagenic fraction through a conjugative--beta-lyase pathway (reviewed by Anders et al., 1988 and DeKant et al., 1989; see also, Section III [Metabolism] of this document). This secondary metabolic pathway, which may assume greater importance with saturation of the oxidative pathway, is initially catalyzed by hepatic cytosolic and microsomal glutathione S-transferases to yield S-(1,2,2-trichlorovinyl) glutathione (TCVG). After transport to the kidney, TCVG is metabolized to S-(1,2,2-trichlorovinyl) cysteine (TCVC) by the enzymatic removal of glutamyl and glycine residues. TCVC is acetylated to N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine, which is excreted in the urine. However, TCVC is also a substrate for renal beta-lyases which cleave TCVC to yield an unstable thiol that may give rise to cytotoxic and mutagenic intermediates.

In vivo and in vitro experiments provide evidence to support this metabolic and cytotoxic/mutagenic scheme. DeKant et al. (1987) and Green et al. (1990) have demonstrated hepatic conjugation of perchloroethylene with glutathione by rodent liver fractions in in vitro experiments. Vamvakas et al. (1989) found TCVG in the bile excreted by

perchloroethylene-perfused rat livers and Green et al. (1990) reported the presence of the glutathione conjugate in the bile of rats administered perchloroethylene by gavage.

Human liver samples have been compared to rat and mouse liver samples with respect to ability to conjugate perchloroethylene with glutathione (Green et al., 1990). These investigators found low levels of conjugation by rat and mouse livers but were unable to demonstrate conjugation by the human livers. To confirm the viability of glutathione S-transferase in the rat and human liver samples studied, these workers compared the two species with respect to their ability to conjugate 1-chloro-2,4-dinitrobenzene with glutathione. Both species carried out this conjugation rapidly and at essentially the same rate. This finding indicates that failure of human liver samples to conjugate perchloroethylene was not attributable to an inactive glutathione S-transferase.

An inability of human liver to conjugate perchloroethylene with glutathione would indicate that toxicologic effects attributable to conjugative metabolites in animals would have little, if any, relevance to human health hazard. However, because of the limited numbers of human livers tested, it is impossible to conclude that humans are unable to carry out this metabolic step. Additional confirmatory studies are clearly needed.

Vamvakas et al. (1989) studied the mutagenicity of chemically synthesized TCVG in a modified Ames protocol employing *Salmonella typhimurium* TA100. In the absence of an exogenous activating system the conjugate produced a weak mutagenic response. In the presence of rat kidney microsomes, mitochondria or cytosolic fractions (sources of gamma glutamyl transferase [GGT] and dipeptidase), TCVG caused marked, dose-related mutagenic responses. These responses were reduced when the protein fraction was pretreated with either a beta-lyase or a GGT inhibitor. A mutagenic response was not observed when hepatic enzymes were used in place of kidney fractions.

The results of these experiments show that TCVG requires metabolic activation to express its marked mutagenic activity. Further, the enzymes required to carry out this activation are found in the rat kidney, not in the liver. This distribution of enzymes is consistent with the production of renal tubule neoplasia in perchloroethylene-treated rats.

Bile, collected from rat livers perfused with perchloroethylene, was found to contain TCVG and, when tested in the Ames protocol using kidney particulate fractions as the

activating system, was clearly mutagenic. As in the experiment with synthetic TCVG, inhibition of renal beta-lyase or GGT reduced the mutagenicity of the bile samples (Vamvakas et al., 1990).

Green et al. (1990) reported the presence of N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine in the urine of rats dosed with perchloroethylene by gavage and rats and mice dosed by inhalation. These investigators have also shown that renal cytosolic beta-lyase from rats, mice, and humans are capable of metabolizing TCVC. Others also have shown that the beta-lyase pathway is present in human proximal tubular cells, and is responsible for activating glutathione and /or cysteine conjugates of a variety of chloro- and fluoroalkenes to reactive metabolites capable of binding to cellular macromolecules (Chen et al., 1990).

In additional studies, TCVG has been found to induce unscheduled DNA synthesis in a porcine kidney cell line and TCVG and N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine have both been found to be mutagenic in the Ames test (DeKant et al., 1989).

The available data indicate that metabolism would be a prerequisite for perchloroethylene mutagenicity. The data do not support classifying the parent compound per se as a mutagen. Although certain metabolites of oxidative metabolism may be mutagenic (e.g., the chloroacetaldehydes including chloral hydrate), these positive data are predominantly limited to in vitro studies. Moreover, perchloroethylene was assayed in the presence of several types of metabolic activation systems (e.g., liver homogenates and intact hepatocytes) that would favor oxidative metabolism and, under these conditions, predominantly negative results were found.

Perchloroethylene may also be activated by a minor pathway involving conjugation with glutathione followed by renal processing of the S-conjugate. This S-conjugate is a beta-lyase dependent mutagen in the *Salmonella*/mammalian microsome assay. Mutagenic metabolites formed in the kidney could conceivably contribute to the tumors observed in male rat kidneys. However, these mutagenicity studies of perchloroethylene metabolites formed by the kidney are in vitro and have been conducted in only one laboratory.

The mutagenicity studies on metabolites of perchloroethylene emphasize the need for further studies concerning a mutagenic role for them in perchloroethylene carcinogenesis.

5. MOUSE LIVER TUMORS

5.1. CARCINOGENICITY BIOASSAY DATA AND EPA'S POSITION

In carcinogenicity bioassays, perchloroethylene has been shown to cause a statistically significant increase in the incidence of hepatocellular carcinoma in both sexes of B6C3F1 mice, following either oral gavage administration or inhalation exposure (NCI, 1967; NTP, 1986).

In a study conducted by NCI (NCI,1976), groups of 50 male mice received time-weighted average doses of 536 or 1,072 mg/kg of perchloroethylene in corn oil by intragastric gavage for 78 weeks (450 or 900 mg/kg for 11 weeks, then 550 or 1,100 mg/kg for 67 weeks). Groups of 50 female mice received time-weighted average doses of 386 or 772 mg/kg of perchloroethylene in corn oil by gavage (300 or 600 mg/kg for 11 weeks, then 400 or 800 mg/kg for 67 weeks). Mice were dosed 5 days per week. The perchloroethylene used in the study was greater than 99% pure, but identification of impurities was not made (NCI, 1976; U.S. EPA, 1985). However, the test sample was estimated to contain epichlorohydrin concentrations of less than 500 ppm (U.S. EPA, 1985). It was considered unlikely, however, that the tumor response resulted from this low concentration of epichlorohydrin. Perchloroethylene caused statistically significant ($p < 0.001$) increases in the incidences of hepatocellular carcinoma in both sexes of mice in both treatment groups when compared to untreated controls or to vehicle controls. The Time to tumor was decreased in treated mice.

Additional studies reported by the NTP confirmed the finding of hepatocellular carcinoma in B6C3F1 mice exposed to perchloroethylene. Groups of 50 mice of each sex were exposed to perchloroethylene concentrations of 0, 100, or 200 ppm by inhalation exposure, 6 hours a day, 5 days per week, for 103 weeks. Perchloroethylene caused dose-related statistically significant increases in the incidences of hepatocellular carcinoma in both sexes.

The biologic significance of chemically-induced mouse-liver tumors, with respect to human hazard identification and the use of such tumor data in assessing cancer risk to humans, is a subject of extensive debate. The controversy surrounding the liver tumor response in the B6C3F1 mouse specifically is well recognized and has been going on for

some time. Several meetings and symposia on the subject have been held and numerous publications have appeared dealing with different aspects of the subject (e.g., Popp, 1984; Stevenson et al., 1990). EPA is aware of the divergent scientific views regarding the predictive validity of mouse liver tumors in the assessment of carcinogenic risk in general, and in the case of perchloroethylene in particular. The EPA undertook an extensive review of the issues concerning mouse liver tumors while it was developing its guidelines for carcinogen risk assessment, and has kept abreast of the issues since that time.

The relevance of mouse liver tumors to the assessment of carcinogenicity in humans has been questioned because of:

- the high, and sometimes variable, background incidence of spontaneously occurring tumors in certain strains of mice, particularly the male B6C3F1 mouse used in the perchloroethylene studies conducted by NCI and NTP;
- the observation that liver cancer is a relatively uncommon cause of death in the United States (although not worldwide); and
- some of the hypothesized mechanisms for mouse liver tumorigenesis that many scientists believe would be unlikely to occur in humans.

On the other hand, many scientists believe that mouse liver tumors are as relevant as any other tumor type observed in laboratory test animals. This later viewpoint concurs with the philosophy of using a sensitive model to detect a response in small numbers of test animals. Also, certain proposed mechanisms, such as oncogene activation, involve steps that are comparable to those observed in the development of other tumor types both in animals and in humans (McConnell, in Stevenson et al., eds., 1990). At least eight of the less than thirty known human carcinogens cause liver tumors in mice, and most of these chemicals also cause other types of tumors in rodents (IARC, 1987).

Hepatocellular tumors are common end points in rodent carcinogenicity studies. Of the chemicals tested in the NTP's bioassay program, 50% of those testing positive caused increased incidences of liver tumors in mice. Most however, also caused other tumors in mice or rats as well (Maronpot et al., 1987; Haseman et al., 1984). Only about 5 to 6% of the compounds studied in NTP carcinogenesis bioassays induced only mouse liver tumors

(McConnell, 1990). Of the chemicals evaluated as carcinogens by EPA, fewer than 10% have been found to cause only mouse liver cancer (Beal, 1990).

At this time, the Agency's position is that increased incidences of mouse liver tumors are considered evidence for human carcinogenic potential, although the evidence may be downgraded on a case-by-case basis according to chemical-specific data. The current EPA policy for evaluating mouse liver tumor data is described in the Guidelines for Carcinogen Risk Assessment, published in 1986 (U.S. EPA, 1986):

> An increased incidence of neoplasms that occur with high spontaneous background incidences (e.g., mouse liver tumors and rat pituitary tumors in certain strains) generally constitutes "sufficient" evidence of carcinogenicity but may be changed to "limited" when warranted by the specific information available on the agent (p 1-7)."
>
> "For a number of reasons, there are widely diverging scientific views about the validity of mouse liver tumors as an indication of potential carcinogenicity in humans when such tumors occur in strains with high spontaneous background incidence and when they constitute the only tumor response to an agent. These Guidelines take the position that when the only tumor response is in the mouse liver and when other conditions for a classification of "sufficient" evidence in animal studies are met (e.g., replicate studies, malignancy; see section IV), the data should be considered as "sufficient" evidence of carcinogenicity. It is understood that this classification could be changed on a case-by-case basis to "limited," if warranted, when factors such as the following are observed: an increased incidence of tumors only in the highest dose group and/or only at the end of the study; no substantial dose-related increase in the proportion of tumors that are malignant; the occurrence of tumors that are predominantly benign; no dose-related shortening of the time to the appearance of tumors; negative or inconclusive results from a spectrum of short-term tests for mutagenic activity; the occurrence of excess tumors only in a single sex (pp.1-5, 1-6).

Thus, in the absence of convincing evidence to the contrary, the Agency considers increased incidences of mouse liver tumors in replicate studies to be "sufficient" evidence of carcinogenicity.

The 1987 EPA paper on the weight-of-evidence classification for perchloroethylene[1] was in keeping with the Agency's Guidelines for Carcinogen Risk Assessment. The EPA paper stated that:

> a strong carcinogenic response has been demonstrated in two separate experiments, in different laboratories, using different routes of exposure, producing similar dose-related responses, increases the weight of the evidence that the response is indicative of a carcinogenic response in animals. While this interpretation can be debated because the response is seen in mouse liver and is accompanied by some non-neoplastic pathology, the confirmatory finding as well as the nature of the response is viewed by many in the science community as "sufficient evidence" of an animal carcinogenic response, as is stated in the Agency's Carcinogen Risk Assessment Guidelines. Additional support for this view comes from the recent deliberations on the classification of perchloroethylene by IARC /(see footnote 5 in the conclusion section)/.... It is the position of the Agency, therefore, that the new inhalation liver tumor data from the NTP study should add to the weight-of-evidence determination for perchloroethylene.

The EPA paper and the most recent letter from the SAB concerning perchloroethylene (SAB, 1988) are consistent regarding statements about the mouse liver tumors. The SAB letter in fact stated that the Board's consensus on the significance of mouse liver tumors was, "that mechanistic explanations are not sufficiently well developed and validated at this time to change EPA's present approach expressed in its risk assessment guidelines for carcinogenicity." The SAB concluded that,

> the generation of mouse liver tumors by chemicals is an important predictor of potential risks to humans. Of the several mechanistic models under consideration (including regenerative hyperplasia, oncogene activation and trihalomethyl radical formation) the one most promising for immediate application to risk assessment is characterized by proliferation of peroxisomes, an intracellular organelle, in the liver."

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[1]The staff paper sent to the SAB as an attachment to the August 3, 1987, letter from EPA's Administrator, written in response to the formal comments submitted to the Agency by the Halogenated Organics Subcommittee regarding the public SAB review of the draft addendum to the HAD on perchloroethylene.

5.2. PEROXISOME PROLIFERATION AND PERCHLOROETHYLENE

Beginning in 1986, additional information regarding the possible link between peroxisome proliferation and liver cancer in B6C3F1 mice exposed to perchloroethylene, has been published (Odum et al., 1988, Green et al., 1986; DeAngelo et al., 1989; Goldsworthy and Popp, 1987). These newer data warrant an evaluation with respect to the interpretation of perchloroethylene mouse liver tumor data as it relates to human health hazard.

A chemically-induced increase in numbers of hepatic peroxisomes, generally referred to as peroxisome proliferation, has been suggested as the underlying mechanism through which perchloroethylene induces hepatocellular carcinomas in B6C3F1 mice (Odum et al., 1988). Carcinomas are proposed to arise as a result of oxidative damage to the cell, possibly at the level of DNA, caused by elevated concentrations of hydrogen peroxide, a peroxisome degradation product. Hydrogen peroxide is normally degraded by a peroxisomal catalase, however the activity of this enzyme does not increase in a parallel fashion with peroxisomes and other peroxisomal enzymes following perchloroethylene exposure. This enzymic imbalance may result in the accumulation of cytotoxic concentrations of hydrogen peroxide. Although DNA is identified as a potential ultimate target of oxidative damage, the mechanism is still described as "epigenetic" or "nongenotoxic." These terms are used in this context to contrast DNA damaging events that are secondary to other effects caused by perchloroethylene from DNA damage produced by a direct, primary interaction of perchloroethylene or its metabolites with DNA.

Assuming that the observed perchloroethylene-induced peroxisomal proliferation and liver hepatocellular carcinomas in the B6C3F1 mouse are related, one course of reasoning supports the hypothesis that the liver tumors could be unique to that species if this is the only mechanism, therefore, decreasing their predictive validity relative to human health hazard. The central points of the supporting rationale are:

- a major metabolite of perchloroethylene, trichloroacetic acid (TCA), is the peroxisome-inducing agent and, therefore, the cancer-causing agent in mouse liver,
- because of a lower rate of metabolism, and metabolic enzyme saturation at relatively low concentrations of perchloroethylene, rats are not as efficient as mice in metabolizing perchloroethylene to TCA, which explains the lack of an

hepatocarcinogenic effect in rats. This also implies that a threshold level of TCA must be exceeded before peroxisomal proliferation and liver cancer can occur,

- humans are even less efficient metabolizers of perchloroethylene than rats,
- human liver cells would probably not respond to the peroxisome-proliferating activity of TCA even if sufficient levels of the chemical could be produced in humans (because when human liver cells are challenged in vitro with TCA at concentrations known to cause peroxisome proliferation in cultured mouse and rat hepatocytes, no evidence of increased peroxisomal activity can be detected), and
- human hepatocytes are also not as responsive as rodents to other known peroxisome proliferators such as the hypolipidemic drugs and phthalate esters.

The conclusion from the above rationale is that humans, because of an inability to generate sufficient TCA levels from perchloroethylene metabolism, and a general unresponsiveness to peroxisome-proliferating agents, are unlikely to show a hepatocellular carcinogenic response to perchloroethylene, assuming this is the mechanism of action in mice.

Many aspects of the above statements are supported to some extent by experimental data.

- TCA has been shown to be a major metabolite of perchloroethylene (Odum et al., 1988); TCA has been shown to cause peroxisome proliferation (as measured by an increase in peroxisomal enzyme activity) in hepatocytes of mice and rats both in vivo and in vitro after short-term exposure (Elcombe, 1985). Further, TCA has been shown to be a hepatocellular carcinogen in the B6C3F1 mouse (Herren-Freund et al., 1987).
- Perchloroethylene oxidative metabolism approaches saturation at lower levels in rats than in mice. Saturation in rats occurs at atmospheric concentrations in excess of 100 ppm (Ikeda et al., 1972). Consequently, at high atmospheric concentrations of perchloroethylene (i.e., > 100 ppm) mice generate relatively more TCA than do rats (Odum et al., 1988). Following 6 hours of inhalation exposure to 400 ppm, the cumulative blood concentrations of TCA in mice were 6 to 7 times greater than concentrations in rats; peak blood levels of TCA were found to be 13-fold higher in mice than in rats.

  These results show that there is a quantitative difference between rats and mice with respect to their abilities to metabolize perchloroethylene to TCA. Such a difference is consistent with the known species variability in responsiveness to the hepatocarcinogenic effects of perchloroethylene. In view of the peroxisome-

inducing activity of TCA in the rat, which for equivalent doses may be even more responsive than the mouse (Elcombe, 1985), and the belief that peroxisome proliferation is a necessary prerequisite to perchloroethylene-induced hepatocellular carcinogenesis, these results also suggest that TCA per se should be hepatocarcinogenic in rats if sufficient blood levels are achieved (Elcombe, 1985). Although a two-year bioassay was stated to be in progress (Elcombe, 1985), EPA is unaware of published studies on the hepatocellular carcinogenicity of TCA in rats.

- Saturation of human perchloroethylene metabolic processes has been reported to occur at perchloroethylene concentrations of approximately 100 ppm to 400 ppm (U.S. EPA, 1985; Ikeda et al.,1972; Ohtsuki et al., 1983). Odum et al. (1988) summarized evidence suggesting that humans exposed to perchloroethylene would be "exposed to lower concentrations of TCA than mice or rats."

- In in vitro experiments conducted to compare rat, mouse and human hepatocytes with respect to susceptibility to TCA-induced peroxisome proliferation, mouse hepatocytes are more responsive than rat, and human hepatocytes have been found relatively unresponsive (Elcombe, 1985).

  If peroxisome proliferation is required for perchloroethylene-induced liver carcinogenesis, reduced human hepatocyte responsiveness to TCA combined with reduced ability to form TCA supports the hypothesis that perchloroethylene is unlikely to be carcinogenic in humans. Inasmuch as this study was based on only two human livers, the evidence cannot be considered persuasive at this point, however. Considerable individual variation in function may be expected to exist between human livers, particularly in specimens from donors who may have been treated with a variety of drugs. More human liver samples need to be examined before a convincing argument can be made.

- There is some evidence that hepatocytes from humans and other primates are relatively unresponsive to a variety of agents which cause peroxisomal proliferation in rodents, although this evidence is limited in quantity and scope. Microscopic studies on liver biopsies from humans chronically dosed with hypolipidemic drugs suggest that these agents do not cause peroxisome proliferation in humans (Cariot et al., 1983; De La Inglesia et al., 1982; Hanefeld et al., 1983). Biochemical data, such as peroxisomal enzyme measurements, have not been reported, however. Nafenopin, a peroxisome inducer in rodents, was inactive in cultured marmoset hepatocytes (Bieri et al., 1988), but the drug did cause increased cell proliferation. Several in vitro studies have also provided suggestive evidence that human hepatocytes are relatively unresponsive to hypolipidemic drugs and phthalate-ester plasticizers (Elcombe and Mitchell, 1986; Butterworth et al., 1989). Here again, however, the sample size is limited to a few human livers. Interpretation of the data is subject, therefore, to the same uncertainty as the in vitro TCA studies on human hepatocytes.

Although evidence does exist to afford support for the hypothesis that mouse liver cancer associated with exposure to perchloroethylene may be secondary to peroxisome proliferation, there are points that run counter to the hypothesis and thus its validity remains questionable. For example:

- If peroxisome proliferation is causally related to the induction of liver cancer, one would expect to detect a quantitative relationship between the two events. That is, potent peroxisome proliferators should also be potent hepatocarcinogens. This does not appear to be the case.

  In a chronic 65-week study of DCA and TCA in male B6C3F1 mice, Herren-Freund et al.(1987) found that DCA was a more potent hepatocarcinogen than TCA. Equidoses (5 g/L in drinking water) resulted in a nearly threefold higher incidence of liver cancer in DCA-dosed animals than in TCA-dosed animals. DeAngelo et al. (1989) however, reported that TCA was more potent as a peroxisome proliferator than was DCA in male B6C3F1 mice. Nelson et al. (1989) also reported that TCA produced greater peroxisome proliferation than did DCA in B6C3F1 mice dosed for only 10 days.

  An even more pronounced lack of correlation was reported in a study of the peroxisome proliferators, DEHP and Wy-14643 (Marsman et al., 1988). At doses producing equivalent increases in peroxisome volume density and peroxisomal enzyme activity for the two compounds, the liver lesions and tumors were produced only by Wy-14643 (100% incidence). DEHP produced no liver lesions in dosed rats.

- Studies on the Swiss mouse also raise questions about the connection between peroxisome proliferation and cancer. If mouse liver peroxisome proliferation in response to perchloroethylene is correlated to the induction of liver cancer, one would expect strains of mice that exhibit peroxisome proliferation to also exhibit hepatocellular carcinoma induction. While the EPA is unaware of perchloroethylene bioassays being conducted in mouse strains other than B6C3F1 and Strain A, the closely related hepatocarcinogen in B6C3F1 mice, trichloroethylene, has been studied in the Swiss mouse (Henschler et al., 1984). Trichloroethylene, which is metabolized to TCA, induces peroxisome proliferation in Swiss mice (Elcombe, 1985). Hepatic peroxisome proliferation was induced as measured by both an increase in peroxisomal enzymes and peroxisome density volume. Induction of the peroxisome marker enzyme, cyanide insensitive palmitoyl CoA oxidase activity, increased linearly with increasing trichloroethylene doses of 0.05-0.5 g/kg/day following 10-day exposure periods (Elcombe, 1985). TCA, a major metabolite of trichloroethylene, also induced peroxisome proliferation in Swiss mice. The daily oral administration of trichloroethylene to Swiss mice for 18 months did not produce liver cancer in either sex, however (Henschler et al., 1984). The doses

administered by Henschler et al. were from 3 to 5 times higher than doses found by Elcombe to induce peroxisome proliferation in these mice.

- The results of recent studies have raised the possibility that genotoxicity may occur independently of peroxisomal proliferation following exposure to perchloroethylene.

  Recent studies have shown that the TCA and DCA metabolites of perchloroethylene have DNA-damaging activity without increasing the levels of peroxisomal enzymes. Within 1 hour after a single dose of TCA or DCA at 0.5 g/kg, a significant increase in single-strand breaks in DNA was detected using an alkaline unwinding assay (Nelson et al., 1989). No increase in peroxisomal palmitoyl-CoA oxidase activity was detected for periods up to 24 hr after dosing. This study raises the possibility that genotoxicity, and thus potential mutagenicity, may occur independently of peroxisome proliferation following perchloroethylene exposure. As discussed earlier, other investigators have been unsuccessful in demonstrating single-strand breaks in DNA with TCA and DCA (Chang et al., abstr., 1989).

  Other metabolites, such as chloroacetaldehydes like chloral hydrate, might also contribute to liver tumorigenesis. A preliminary study in mice indicates that chloral hydrate is a hepatocarcinogen in mice (Rijhsinghani et al., 1986;). Chloral hydrate has been shown to produce aneuploidy and may be mutagenic (see Section 4.2.).

- More recently, Nelson et al. (1990) reported that TCA administration significantly increased expression of the C-H-ras and c-myc oncogene in hepatocellular carcinomas in B6C3F1 mice. The data indicate that elevated expression of these oncogenes may play an important role in the development of liver tumors in these mice. As discussed by these authors, the consequence of increased c-myc expression in most cells is loss of cellular differentiation. The oncogene does not induce cell division, but appears to play a permissive function in relation to cell division. These workers suggest that TCA increased c-myc expression may prevent initiated cells from differentiating, thereby increasing their probability of progressing to hepatocellular carcinoma. Several investigators have reported a different pattern of oncogene activation in chemically induced mouse liver tumors compared to that observed in the tumors of untreated animals. This indicates that the chemicals do not simply promote spontaneous background tumors (Fox et al., 1990; Reynolds et al., 1987).

In summary, there is some evidence to support the hypothesis that perchloroethylene-induced hepatic carcinogenesis may be related to peroxisome proliferation. However, critical review of the scientific literature reveals significant data gaps regarding the relationship between the proliferative effect and neoplasia. The recent demonstration of a peroxisome-

proliferator-activated receptor (Issemann and Green, 1990) should lead to increased understanding of the mechanism of action for chemicals causing this phenomenon. Also, the recent demonstration, that the major metabolite of perchloroethylene, TCA, causes the expression of the c-myc oncogene in B6C3F1 mice, requires experimental exploration.

6. KIDNEY TUMORS IN MALE RATS

The inhalational administration of perchloroethylene to male and female F 344/N rats and B6C3F1 mice produced dose-related increases in the incidences of nontumor nephrotoxicity in both sexes of both species and a nonstatistically significant increase in the incidence of proliferative lesions of the renal tubular cells (tubular cell hyperplasia, adenoma, and adenocarcinoma) in male rats (NTP, 1986). A slight increase in renal tumors was also observed in male Sprague-Dawley rats receiving perchloroethylene by gavage or by inhalation in other studies (Maltoni and Cotti, 1986; Rampy et al., 1978).

In the NTP studies groups of 50 male and 50 female F 344/N rats were exposed by inhalation to atmospheres containing 0, 200, or 400 ppm perchloroethylene for 6 hours a day, 5 days per week for 103 weeks. Tubular cell hyperplasia, was observed in male rats (control 0/49, low dose 3/49, and high dose 5/50) and in one high dose female. Renal tubule neoplasms were observed in male rats (control 1/49, low dose 3/49, and high dose 4/50).

Although the incidences of renal tubule neoplasms in perchloroethylene exposed male rats was not statistically significant ($p > 0.05$) relative to concurrent controls, the production of the lesions is considered to be evidence of a carcinogenic effect in rats. This is supported by the following facts:

- Renal tubule tumors occur only rarely in F 344/N rats. Historically, the NTP has found renal tubule neoplasms in only 0.2% of male F 344/N rats (chamber controls from the performing laboratory, 1/249 (0.4%) and untreated controls from non-inhalation studies, 4/1968 (0.2%)). Likewise, the overall historical control incidence of renal tubule tumors in male F 344/N vehicle controls in gavage studies is 1/1943 (0.05%). The incidence is even lower in female controls. This is supported by spontaneous renal tubule tumor incidence rates recorded for other rat strains (e.g., Osborne-Mendel, males 0.3%; females 0%; Goodman et al., 1980). The appearance of tubule neoplasms in 7% of perchloroethylene-dosed animals (low- and high-dose groups combined) is convincing evidence of a treatment-related effect.

- No malignant renal tubule neoplasms have been observed in any control rats examined by the NTP. This includes the chamber controls from the performing laboratory, and the untreated controls and the vehicle controls from gavage studies. Two of the tumors observed in high-dose animals in the NTP study were

carcinomas. The probability of two rare carcinomas appearing by chance in a group of 50 animals has been calculated to be less than 0.001 (U.S. EPA, 1987).

- When statistically compared to historical control incidences of renal tubule tumors, there is a significant dose-related positive trend, and tumor incidences in both low- and high-dose groups are significantly elevated. Although standard statistical analyses of tumor incidence data did not reveal a significant increase in kidney tumors, when the incidences of tubular cell hyperplasia (0/49, 3/49, 5/50) and neoplasms (1/49, 3/49, 4/50) and tumor severity (two adenocarcinomas in the high-dose group) are all considered, a dose-response relationship is apparent. Thus, although the tumor incidence is not statistically significant when compared with concurrent controls, there is a positive trend.

In addition to the NTP study findings of renal tubule tumors in male F 344/N rats, Rampy et al. (1978) and Maltoni and Cotti (1986) reported slight increases in renal tumors in Sprague-Dawley rats dosed with perchloroethylene by inhalation and gavage, respectively.

There is good evidence that the tubule tumors are not unique to the administration of perchloroethylene. The NTP has found low incidences of tubule neoplasms in rats dosed with other chlorinated ethanes and ethylenes (NTP, 1983, 1988; and unpublished results cited in NTP, 1986). There is some evidence that nontumor pathology is not unique to perchloroethylene; however, there is also evidence that the nephrotoxicity observed with certain chemicals of this group, such as pentachloroethane, may be different from that seen with others of these compounds, such as trichloroethylene.

The data support the conclusion that the chronic administration of perchloroethylene produces nephrotoxicity in both sexes of mice and rats and an increased incidence of proliferative lesions of the kidney tubules in male rats. The use of these data to infer risk of carcinogenesis to humans, however, is a focus of scientific debate. Of particular consequence in this debate is the possibility that the induction of renal tubule tumors by perchloroethylene may be unique to male rats, and therefore, is inappropriate for deducing potential human health hazard. This is because reasonable evidence exists to suggest that renal effects induced in male rats by chemicals causing alpha-2μ-globulin accumulation are unlikely to occur in any species not producing alpha-2μ-globulin or a protein with a structurally similar binding domain, in the large quantities typically seen in the male rat. Thus, if a chemical induces alpha-2μ-globulin accumulation in hyaline droplets, and a carcinogenic

response in the male rat kidney, the tumor response may not constitute evidence of a carcinogenic hazard to humans.

The EPA is presently developing criteria which will define a weight-of-evidence approach for evaluating, on a case by case basis, the role of alpha-2μ-globulin in rat kidney tumor formation (U.S. EPA, 1991). A report (U.S. EPA, 1991) currently being developed by a technical panel of EPA's Risk Assessment Forum, provides guidance on determining when it is reasonable to presume that a renal tumor in male rats results from alpha-2μ-globulin accumulation, and on selecting appropriate procedures to use in extrapolation to humans under such circumstances. The report also defines other situations that suggest a different approach and calls for research to clarify questions raised because of the existence of human proteins that may be structurally similar to alpha-2μ-globulin. Data on renal tumors in the male rat will fall into several categories depending on whether the tumors are attributable solely to alpha-2μ-globulin accumulation, whether another mechanism applies, whether several mechanisms are feasible, one of which involves alpha-2μ-globulin, or whether the available information is inadequate to determine the role of alpha-2μ-globulin. For instance, if the perchloroethylene alpha-2μ data are subsequently judged to be the only definitive explanation for the occurrence of male rat kidney tumors, this tumor end point may not have relevance for human health hazard assessment. This can be further evaluated as the EPA's criteria for identifying chemicals inducing alpha-2μ-globulin accumulation become available to apply to the perchloroethylene-specific data.

6.1. ALPHA-2μ-GLOBULIN IN RENAL CARCINOGENESIS IN MALE RATS

A variety of organic compounds studied by the NTP and others have been shown to produce sex- and species-specific lesions in the renal tubules of male rats in the form of hyaline droplet nephropathy (NTP, 1987, 1986, 1988, 1983; Alden et al., 1985; MacNoughton and Uddin, 1984; Alden et al., 1984; Phillips et al., 1987). The accumulation of the protein, alpha-2μ-globulin, is believed to be the reason for an excessive number of hyaline droplets (Stonard et al., 1986; Olson et al., 1987). A normal urinary protein in the male rat, alpha-2μ-globulin is synthesized in the liver under hormonal control but it has not been detected in the liver of female rats nor in other species, including humans (HEI, 1988). Among the chemicals

tested so far in chronic animal bioassays, those that invoked this specific type of protein droplet nephropathy in male rats also produced renal tubule tumors in male rats, but did not produce renal tubule tumors in other species tested. The renal tubule tumors appear to be the end product in the following sequence of functional changes in the epithelial cells of proximal tubules (UAREP, 1983; Alden et al., 1984 Halder et al., 1984; HEI, 1988; Swenberg et al., 1989):

- Excessive accumulation of hyaline droplets in proximal tubules, representing lysosomal overload, leads to tubule cell degeneration, cell loss, and regenerative cellular proliferation.
- Cell debris in the form of granular casts accumulates at the corticomedullary junction with associated dilation of the affected tubule segment and more distally, mineralization of tubules within the renal medulla.
- The chronic progressive nephropathy characteristically found in aging rats is exacerbated as a consequence of the induced nephrotoxicity.
- Renal tubule hyperplasia and neoplasia develop subsequently.

A number of investigators hypothesize that the increased proliferative response caused by the chemically-induced cytotoxicity results in clonal expansion of spontaneously initiated renal tubule cells and increased incidence of renal tumor formation (Trump et al., 1984; Alden, 1989; Swenberg et al., 1989). This line of reasoning leads to a conclusion that the acute and chronic renal effects induced in male rats by these chemicals will be unlikely to occur in any species not producing alpha-2μ-globulin, or a very closely related protein, in the large quantities typically seen in the male rat (Alden, 1989; Borghoff et al., 1990; Green et al., 1990; Olson et al., 1990; Flamm and Lehman-McKeeman, 1991).

This proposed mechanism of tumorigenesis seems plausible and may provide an adequate explanation of the specific susceptibility of the male rat to the induction of renal tubule tumors by certain chemicals. However, definitive links between alpha-2μ-globulin accumulation and tumorigenesis in male rats must be established on a chemical-by-chemical basis before it is reasonable to discount the significance of the tumor induced by a particular

chemical. Guidance for evaluation of data concerning alpha-2μ-globulin and kidney tumors in male rats is being developed by EPA (U.S. EPA, 1991).

Goldsworthy and his coworkers (1988) observed increases in alpha-2μ hyaline droplets and crystalloid accumulation in the cytoplasm of the P2 segment of proximal tubules of male, but not female, F 344/N rats, following 10 days of gavage with 1000 mg/kg perchloroethylene. Cell replication was enhanced in the male rats, specifically in damaged P2 segments, suggesting a link between the alpha-2μ-globulin accumulation and kidney tumors. These investigators reported similar findings for pentachloroethane, but at a dose of 150 mg/kg for 10 days. Trichloroethylene, structurally very closely related to perchloroethylene, was not found to cause an increase in protein droplets or cell replication in either male or female rats administered 1000 mg/kg for 10 days.

In short-term, high-dose studies, Green et al. (1990) found that the oral administration of from 1000 to 1500 mg/kg of perchloroethylene daily for up to 42 days caused an accumulation of alpha-2μ-globulin in the proximal tubules of male rats. The animals were killed within 24 hours of the last dose of perchloroethylene. The effect was accompanied by evidence of nephrotoxicity, with the formation of granular tubular casts and evidence of tubular cell regeneration. The effects were not observed in female rats or in mice. Inhalation exposure to 1000 ppm of perchloroethylene for 10 days resulted in the formation of hyaline droplets in the kidneys of male rats, but granular casts and tubule cell regeneration were not observed, although the time period may have been too short. These results show that very high doses of perchloroethylene are capable of precipitating hyaline droplet nephropathy in male rats and that male rats are far more sensitive to the effect than are female rats or either sex of mice. Therefore, alpha-2μ-globulin accumulation may play a role in the tumorigenesis observed in male rats exposed to perchloroethylene.

The following points, however, show that factors other than the specific protein droplet nephropathy may have as much or more of a significant role in explaining renal tumor formation resulting from perchloroethylene exposure, although some contribution of alpha-2μ-globulin accumulation cannot be ruled out.

The Alpha-2μ-Response for Perchloroethylene is Relatively Mild, and Renal Tumors Have Been Observed at Doses Lower than the Ones Shown to Cause the Alpha-2μ-Response.

Although the alpha-2μ-response does occur in male rats exposed to perchloroethylene, it has been observed following only high doses. While Green et al. (1990) tested lower inhaled doses of perchloroethylene (up to 400 ppm 6 hours per day for 28 days with animals being sacrificed within 18 hours of termination of the final exposure) in rats, there was no evidence of hyaline droplet formation although there may have been time for recovery before sacrifice. It is noteworthy that the 400 ppm concentration was the same exposure level used for the high-dose rats in the NTP inhalation carcinogenicity bioassay. In the NTP study the 400 ppm concentration caused a high incidence of non-tumor nephropathy and resulted in the formation of kidney tubule adenomas and adenocarcinomas. The renal pathology of rats in the NTP study was reported to be different from the specific alpha-2μ-nephropathy, but the age of the rats, as well as the length of time that elapsed between final exposure and sacrifice, may explain some of the differences. Mineralization in the inner medulla and papilla of the kidney, a characteristic trait of alpha-2μ-nephropathy, was not seen, however (NTP, 1986).

It is possible that longer-term exposure to the 400 ppm concentration of perchloroethylene is required for the production of hyaline droplet accumulation in the kidney of rats (ECETOC, 1990). Alpha-2μ-globulin accumulation can be demonstrated, however, after only short-term exposures (even a single administration) to several agents such as d-limonene, decalin, unleaded gasoline, and trimethylpentane (Charbonneau et al., 1987; NTP, 1988). Lack of hyaline droplet formation or increase in alpha-2μ-globulin or signs of the characteristic renal nephropathy at the high-dose level of the NTP inhalation study may indicate a threshold effect and thus diminish the likelihood that the renal tumors associated with exposure to perchloroethylene are induced through this mechanism (Green, 1990). Pharmacokinetic differences between oral and inhalation exposure may contribute to the observed discrepancies in some of the results.

The NTP did not report the presence of hyaline droplets in rats that had been exposed to either 200 or 400 ppm of perchloroethylene for up to 2 years. These doses were associated with the production of renal tubule neoplasms in male rats. The fact that the NTP

did not report the presence of hyaline droplets in either the 14-day, 90-day, or 2-year studies is not definitive, however, since the NTP protocol at that time was not designed to detect hyaline droplets or alpha-2μ-globulin accumulation in the kidney (NTP, 1990). Thus, the procedures followed at the time of the study were not necessarily conducive to detection of hyaline droplets. For example, in the chronic study of perchloroethylene, at least one week elapsed between the final perchloroethylene exposure and the scheduled sacrifice of the surviving animals. It is possible that had the hyaline droplets been present they could have regressed. Also, the nephropathy observed at the end of a 2-year bioassay could be difficult to distinguish from the old-age nephropathy that occurs in these rats. Other investigators (Goldsworthy et al., 1988; Green et al., 1990) have observed hyaline droplets containing alpha-2μ-globulin following high doses of perchloroethylene administered to male rats.

In the NTP bioassay, however, the renal pathology reported is not entirely consistent with the results generally found for chemicals where there is alpha-2μ accumulation (NTP, 1986; letter from Scot Eustis to William Farland, 1988). For example, as mentioned above, there was no mineralization in the inner medulla and papilla of the kidney, a frequent finding in bioassays of chemicals inducing alpha-2μ-globulin accumulation (e.g., for pentachloroethane, the incidence of renal papillar mineralization is 8% in controls; 59%, low dose; 58%, high dose). In addition, some aspects of toxic tubular nephropathy were also observed in female rats and male mice exposed to perchloroethylene.

Perchloroethylene does induce alpha-2μ-globulin accumulation and some of its associated nephropathy in male rats, although the evidence for this exists only at high doses. Nevertheless, the hypothesis of hyaline droplet formation leading to renal tubule tumors in male rats is a valid proposal for mechanisms of tumorigenesis. The absence of evidence that chronic inhalational exposure to 200 or 400 ppm of perchloroethylene causes the accumulation of hyaline droplets and its associated nephropathy in the kidneys of male rats, considered along with the data supporting other mechanisms, including possible genotoxicity discussed below, makes it difficult to conclude, however, that perchloroethylene-induced renal tumors can be attributed solely to this hypothesized species/sex specific mechanism.

Chronically-Induced Perchloroethylene Nonneoplastic Kidney Lesions Exhibit Neither Species Nor Sex Specificity.

In contrast to most other chemicals inducing alpha-2μ-globulin accumulation that have been tested by NTP in chronic carcinogenicity bioassays, renal lesions occurring in animals exposed to perchloroethylene were not confined to the male rat. Although the female rat did not develop any renal tubule tumors, the incidence of karyomegaly was significantly elevated in the female rat as well as in the male rat; one of 50 female rats exposed at the high dose developed tubular cell hyperplasia.

In the mouse, "nephrosis" was observed at increased incidences in dosed females, casts were observed at increased incidences in dosed males and high-dose females, and karyomegaly of the tubular cells was observed at increased incidences in both sexes of treated mice. The severity of the renal lesions was dose related and one low-dose male had a renal tubular cell adenocarcinoma.

In the NCI gavage study of perchloroethylene, toxic nephropathy, not detected in the control animals, occurred in both male and female Osborne-Mendel rats administered perchloroethylene. Unfortunately, the animal survival in this study was not adequate to support any conclusions about perchloroethylene carcinogenicity.

Other chlorinated ethanes and ethylenes produce nephrotoxicity and renal tubule tumors in laboratory animals as well. Hexachloroethane causes accumulation of hyaline droplets and renal tubule tumors in male rats. On the other hand, trichloroethylene, for example, which was also tested by NTP, induces kidney tumors in male rats only (NTP, 1988b) but does not cause an accumulation of hyaline droplets or an increase in levels of alpha-2μ-globulin (Goldsworthy et al., 1988). Consequently, kidney tumors induced by this compound are not considered to be associated with alpha-2μ accumulation. Perchloroethylene is closely related structurally to trichloroethylene, and both of these chemicals have been shown to be metabolized in the kidney to mutagenic compounds.

6.2. SUSTAINED CHRONIC NEPHROTOXICITY AS A POSSIBLE MECHANISM INDEPENDENT OF ALPHA-2μ-GLOBULIN ACCUMULATION

Numerous compounds such as perchloroethylene, trichloroethylene, and pentachloroethane have been reported to produce nephrotoxicity in male and female rats and mice. This toxicity, although appearing to be characteristic of chronic administration of chlorinated ethanes and ethylenes, manifests itself differently with specific ones of these chemicals and may include tubular cell cytomegaly, karyomegaly and pleomorphism, tubular cell dilation, or the formation of granular casts. Certain of these compounds cause kidney tumors in male mice only (vinylidene chloride), male rats only (trichloroethylene), and both male rats and male mice (chloroform).

As previously discussed for the alpha-2μ-nephropathy, sustained kidney damage may be a risk factor for tumorigenesis. Thus, there may be a link between renal toxicity and tumorigenesis, and it is reasonable to suspect that renal tubule neoplasia in male rats may be influenced by perchloroethylene-induced cytotoxicity and subsequent cellular regeneration. It also has been suggested that renal neoplasms induced by perchloroethylene may be secondary to renal cytotoxicity and subsequent cellular proliferation without regard to alpha-2μ-globulin accumulation. If this is the case, renal tubule neoplasia in these experiments would not be expected to be a species/sex-specific response to chronic administration of perchloroethylene because the nontumor lesions appeared in both sexes of both species. Perchloroethylene-induced cytomegaly and karyomegaly appeared in both rats and mice during the early phases of the NTP inhalation study indicating that animals of both species surviving to the scheduled termination of the study had long-standing nephrotoxicity. If renal tubule neoplasia were directly consequent to this pathology, tumors would likely have been found in dosed female rats or male and female mice. Goldsworthy et al. (1988) determined that cell-replication rates increased specifically in the histologically damaged tubule segments of male rats, but not in female rats, after perchloroethylene exposure. Cell replication did not differ from controls in trichloroethylene-treated male or female rats, however. Since both trichloroethylene and perchloroethylene produce renal tubule tumors, but no enhanced cell replication was seen with trichloroethylene, it is difficult to conclude that perchloroethylene

Induces the renal tumors by a nephrotoxic mechanism apart from nephropathy associated with alpha-2μ-globulin accumulation.

The fact that there is little doubt that the kidney is a target organ for perchloroethylene and other chlorinated ethanes and ethylenes in mammalian species contributes to the overall concern regarding the kidney tumor end point. Although nephrotoxicity may play a role, more supportive evidence is needed to define such a role.

6.3. A MUTAGENIC MECHANISM OF PERCHLOROETHYLENE-INDUCED CARCINOGENESIS IN MALE RATS

The possible explanations of perchloroethylene-induced renal carcinogenesis discussed earlier have centered on nonmutagenic (epigenetic) mechanisms. This is because mutagenicity studies of perchloroethylene have produced largely negative or only weakly positive results. The early studies of genetic toxicology of perchloroethylene have centered on the effects of perchloroethylene per se and later on certain of its products of oxidative metabolism. A secondary metabolic pathway for perchloroethylene (hepatic conjugation with glutathione and subsequent degradation by renal beta lyases) has been discovered in rats (see sections III and IV on metabolism and mutagenicity; also see Dekant et al., 1989; Vamvakas et al., 1989). Perchloroethylene is conjugated with hepatic glutathione to form S-(1,2,2-trichlorovinyl) glutathione (TCVG). The conjugative pathway in the liver appears to be the minor of two competitive pathways; its activity is thought to increase as the oxidative pathway approaches saturation. The glutathione S-conjugate metabolite thus formed in the liver is either excreted into the bile or transported to the kidney where it is acted upon by gamma glutamyl transferase and dipeptidase to form its corresponding cysteine S-conjugate, S-(1,2,2-trichlorovinyl)-l-cysteine (TCVC) (DeKant et al., 1987). TCVC may undergo N-acetylation and be excreted in the urine, or it may become a substrate for renal beta-lyases which cleave TCVC to form a mutagenic fragment that probably includes electrophilic acylating and alkylating agents. The mutagenic activities of TCVG, in the presence of hepatic- and renal-activating systems, and TCVC, in the presence of a renal-activating system, have been demonstrated in an Ames test protocol. The conjugative pathways producing mutagenic metabolites in the kidney are operative for trichloroethylene, a close structural analog of

perchloroethylene (Anders et al., 1988; DeKant et al., 1989). Trichloroethylene also induces renal tumors in male rats, but is not a chemical that induces alpha-2μ-globulin accumulation (NTP, 1988b; Goldsworthy et al., 1988; U.S. EPA, 1991). The trichloroethylene conjugates that lead to mutagenic constituents are dichlorovinylglutathione and dichlorovinylcysteine (Anders et al., 1988; DeKant et al., 1989).

Although the beta-lyase enzymes necessary for the metabolism to the mutagenic metabolite are found in the kidneys of rats, mice, and humans (Green et al., 1990; Chen et al., 1990), in vitro conjugation of perchloroethylene with glutathione by human liver was not detected (Green et al., 1990). If the human is incapable of conjugating perchloroethylene with glutathione, this potential mechanism of carcinogenesis may not be relevant to projecting human health hazards associated with perchloroethylene.

Green et al., (1990) have reported species differences with respect to perchloroethylene-glutathione conjugation. In vivo conjugation by rat liver occurred at a relatively low rate but this rate was five times greater than that observed for mouse liver. Using a limited number of human liver samples, Green et al. were unable to demonstrate conjugation of perchloroethylene. Few liver samples were studied, however, and a conjugation rate tenfold lower than that observed for rats would fall below the limits of detection of the method employed. Tenfold differences in enzyme activities within the human population are not uncommon. Consequently, it remains a distinct possibility that humans may conjugate perchloroethylene, although probably at a very low rate as indicated by the low rate measured in rodent tissue.

The roles of this metabolic pathway in the production of renal tumors in male rats and in the carcinogenic potential of perchloroethylene in humans remain to be established. It has been pointed out that the conjugative pathway is minor and may be noticeably more active only when the oxidative pathway approaches saturation. Human (and rat) oxidative metabolism of perchloroethylene is recognized as being saturable. The quantitative relationships between various degrees of saturation of the oxidative pathway and concomitant importance of the conjugative pathway requires close scrutiny before it can be concluded that conjugation is unimportant in perchloroethylene metabolism by humans, particularly since products include potential mutagens.

Also, if the glutathione/beta-lyase pathway provides a mechanism for the induction of renal tumors it is difficult to explain why female rats and both sexes of mice did not exhibit renal tumors. The metabolic processes required for the generation of the mutagenic intermediate are operative in both sexes of both species, albeit to a lesser extent in female rats and both sexes of mice. The male rat tumor rate was relatively low, but the incidences in female rats or male and female mice might be expected to be still lower. The rates in female rats and both sexes of mice might be too low to be detected with the small numbers of animals subjected to testing.

In summary, since the NTP discovery that chronic administration of perchloroethylene induces a low level of renal tubule tumors in male rats, significant research has been conducted to explain the mechanism of the carcinogenic effect. This research has resulted in at least three possible explanations:

1. The tumors may be secondary to the renal accumulation of the low molecular weight protein, alpha-2μ-globulin. Since only male rats produce the protein, the tumors would have little or no predictive validity with respect to human health hazard on a site- or mechanism-specific basis. Perchloroethylene induces kidney tumors at lower doses than those required to cause alpha-2μ-globulin accumulation, however. The EPA is presently developing criteria which will define a weight-of-evidence approach for evaluating, on a case by case basis, the role of alpha-2μ-globulin in rat kidney tumor formation (U.S. EPA, 1991).
2. The chronic administration of perchloroethylene produces nephrotoxicity and it has been suggested that tumor production is secondary to sustained cytotoxicity and cellular regeneration. Although certain "nephrotoxicity" occurs in both sexes of rats and mice, implying that kidney tumors would occur in rats and mice of both sexes in the carcinogenicity bioassays, cell replication occurs in male but not in female rats suggesting that any nephrotoxic mechanism would likely be associated with alpha-2μ-globulin accumulation. On the other hand, the mechanism of perchloroethylene tumorigenesis may be similar to that of its structural analog, trichloroethylene. Trichloroethylene induces kidney tumors in male rats, but does not enhance cell replication and does not cause alpha-2μ-globulin accumulation.

3. A glutathione-beta lyase conjugation pathway of perchloroethylene metabolism has been discovered in rats and also shown in mice. This minor pathway leads to the formation of a cytotoxic/mutagenic metabolite product. It is interesting to note that the structural analog of perchloroethylene, trichloroethylene, forms a cytotoxic/mutagenic metabolite via this pathway and also causes kidney tumors in male rats; but trichloroethylene does not cause alpha-2μ-globulin accumulation. Humans may or may not have the capacity to carry out the initial conjugation step. If the human cannot form the perchloroethylene-glutathione conjugate, this pathway is irrelevant with respect to human risk projection.

While there is some evidence to support each of the proposed mechanisms, there are also significant quantitative and qualitative gaps in the supportive data. The mode of perchloroethylene-induced renal tumorigenesis in male rats is not yet understood.

7. MONONUCLEAR CELL LEUKEMIA IN RATS

The NTP (1986) reported that the chronic inhalational administration of perchloroethylene to male and female F 344/N rats caused positive trends in the incidence of mononuclear cell leukemia (MCL) in both sexes. Pairwise comparisons of tumor incidences in dosed and control groups of males (life table analysis) revealed statistically significant increases in both the low and high dose groups (controls, 28/50; low dose, 37/50; $p = 0.046$: high dose, 37/50, $p = 0.004$; trend test $p = 0.004$). Analysis of the data for female rats revealed a marginally significant trend ($p = 0.053$) and a significant increase in the low-dose group and a marginally significant increase in the high-dose group (control, 18/50; low dose, 30/50, $p = 0.023$; high dose, 29/50, $p = 0.053$).

Interpretation of these data is somewhat clouded by the fact that overall incidences of MCL in the concurrent chamber control groups were high relative to historical chamber control groups at the performing laboratory (males 28/50, 56% versus 117/250, 47%; females 18/50, 36% versus 73/249, 29%). The concurrent control group rates were also higher than the NTP Program historical rate for untreated control groups (males 583/1,977, 29% and females 375/2,021, 18%).

Because of these factors the NTP conducted supplemental analyses of the progression of the disease, the effect of perchloroethylene on the time of onset of advanced MCL, and the contribution of MCL to early deaths in control and dosed animals. The results of these supplemental analyses showed that:

- In both males and females, perchloroethylene produced a dose-related increase in the severity of MCL.
- Perchloroethylene exposure significantly shortened the time to onset of MCL in female rats.
- Although there was no remarkable effect of perchloroethylene exposure on survival of female rats, there was an increased incidence of advanced MCL in female rats that died prior to the scheduled termination of the study. Thus, a more appropriate statistical analysis was conducted in which only the incidences of advanced MCL in

rats were considered. Significantly positive trends and significant increases in the incidences of advanced MCL in both male and female rats in the high-dose groups were observed.

In 1987 the EPA Science Advisory Board took exception to the use of these special analyses since they did not represent generally accepted approaches to the evaluation of increased incidences of MCL. According to the NTP report, however, the interpretation of MCL incidences in the perchloroethylene study was based on standard methods of data evaluation (NTP, 1986). The special analyses were conducted to support, rather than establish, the interpretation.

Under the conditions of the NTP study there was evidence of a carcinogenic effect of perchloroethylene in male and female rats as evidenced by significant increases in the incidences of MCL in both sexes. However, the usefulness of increased incidences of MCL in the prediction of human carcinogenic risk associated with exposure to perchloroethylene has been questioned on several grounds:

<u>MCL is a Common and Variable Tumor that Occurs Spontaneously in F 344/N Rats. Marginal Increases in Incidences are of Questionable Biological Significance.</u>

MCL is recognized as a common neoplasm in rats and its rate of appearance in historical control groups is highly variable. In the five contemporary inhalation studies conducted at the performing laboratory the incidences of MCL in chamber control male rats ranged from 32 to 68% In female chamber control rats the incidences ranged from 22 to 36%. A similarly high variability has been observed among NTP Program-wide untreated control groups (males, 10 to 60%; females, 6 to 38%).

Concurrent controls represent the most appropriate groups to use for the purpose of determining the statistical significance of observed differences between experimental groups. However, it is recognized that in the case of spontaneous and highly variable tumors, comparisons of treatment groups to historical control groups may be helpful in the interpretation of experimental results. When the overall rates of MCL in male rats in the perchloroethylene studies are compared to the range of tumor incidences in historical controls, the perchloroethylene-treated animals were essentially identical to those in the historical

control group with the highest incidence (74% in perchloroethylene-dosed animals versus 68% in the historical control group). However, the incidences of MCL in perchloroethylene-dosed female rats (60 and 58%) were elevated relative to the highest incidence in historical control groups (38%).

The Pathobiology of MCL is Too Poorly Understood to Allow the Tumors to be Used to Determine Human Health Risk.

MCL is a relatively well defined and understood rodent neoplasm. The disease per se, which is splenic in origin, is readily and unequivocally diagnosed by the use of standard histopathological techniques. MCL is known to be a rapidly progressing and fatal neoplasm whose incidence is age related. The tumor is transplantable; and its etiological factor is unknown. It has been suggested that a cellular oncogene may be responsible for the induction of MCL.

Although the specific mechanism of leukemogenesis in rats is not understood, it is interesting to note early reports of toxicity of cysteine S-conjugates where S-(1,2,-dichlorovinyl)-L-cysteine was implicated in induction of aplastic anemia and marked biochemical alteration of DNA in bone marrow, lymph nodes, and thymus in calves (McKinney et al., 1957; Schultz et al., 1959; Bhattacharya and Schultz, 1971, 1972). As discussed earlier, the glutathione conjugate of perchloroethylene is hydrolyzed in the kidney to the cysteine S-conjugate, a compound that can be cleaved to form a mutagenic fragment. Humans as well as rodents activate the conjugate via the b-lyase pathway. Thus, the possibility exists that the perchloroethylene S-conjugate, S-(1,2,2-trichlorovinyl)-L-cysteine, may be involved in the leukemia induction in rats and may have the potential to produce blood dyscrasias in humans as well.

MCL is a Rodent Specific Tumor with no Human Correlate.

MCL is a neoplasm whose incidence and progression can be influenced by chemical agents. While human leukemias originate in bone marrow, MCL is splenic in origin. Discounting a rodent neoplasm simply because it has no exact human counterpart is not a scientifically defensible reason, however. Site concordance is not a requirement for relevancy

in extrapolation of hazard potential, although its actual presence can strengthen belief in a particular hazard e.g., many aromatic amines are probable bladder carcinogens in humans but are likely to produce Zymbal gland tumors rather than bladder tumors in rats. The human does not develop Zymbal gland tumors.

8. SUMMARY AND CONCLUSIONS

The Office of Health and Environmental Assessment has reviewed the data currently available regarding perchloroethylene carcinogenicity. Because the human epidemiology data are inadequate for determining the carcinogenicity of perchloroethylene, the focus of this paper is on the animal data and other information as they are relevant to potential cancer-causing activity in humans. Two questions now must be considered: first, whether the data are judged as "sufficient" evidence of carcinogenicity in animals, and second, whether the general assumption that "sufficient' animal data leads to an overall weight-of-evidence classification of B2, probable human carcinogen, holds up in the case of perchloroethylene.

Is There Sufficient Evidence of Carcinogenicity in Animals?

EPA's Guidelines for Carcinogen Risk Assessment provide criteria to follow in weighing the scientific evidence. The Guidelines also permit the exercise of professional judgment throughout the process, with explanations for the judgment calls. The positive animal evidence for perchloroethylene[2] clearly meets the criteria for "sufficient" animal data as depicted in the Guidelines. Sufficient evidence of carcinogenicity indicates that there is an increased incidence of malignant tumors or combined malignant and benign tumors in (1) multiple species or strains; in (2) multiple experiments (e.g., with different routes of administration or using different dose levels); or (3) to an unusual degree in a single experiment with regard to high incidence, unusual site or type of tumor, or early age at onset.

[2]Perchloroethylene has been shown to cause multiple tumor end points--hepatocellular carcinomas in both sexes of mice, kidney tumors and some indication of gliomas in male rats, and leukemias in both male and female rats. The incidences of liver tumors in mice and the leukemias in rats are statistically significantly elevated when compared to controls. The incidence of renal tumors and gliomas in male rats is significantly elevated when compared to historical controls, and these tumors are biologically significant because they are rare tumors. Carcinogenesis has been demonstrated by both inhalation and oral routes of exposure.

The perchloroethylene data meets, at least to some degree, each of the three independent criteria for attaining "sufficient" animal evidence[3,4]. More robust responses in the rat would add strength to the concern for the response seen in that species, however, the perchloroethylene mouse liver tumor data alone are believed by some to meet the Guidelines criteria for "sufficient" animal evidence since there are multiple experiments having different exposure routes and no appreciable downgrading of the evidence according to the Guideline provisions for mouse liver tumor responses. The leukemia response in both male and female rats, while not momentous, is nevertheless a second and valid end point. The kidney and perhaps glioma responses in male rats are also suggestive and add to having a response in a second animal species which in turn is supportive of the sufficient evidence call. Mutagenicity data are primarily negative for the parent compound, however, metabolism seems to produce mutagenic metabolites.

Does Sufficient Animal Data Indicate Human Hazard?

Ordinarily, animal data labeled as "sufficient," as are the data that exist for perchloroethylene, would be highly indicative of potential human hazard and would lead to an overall weight-of-evidence classification of B2, probable human carcinogen, when considered with the "inadequate" epidemiologic data (EPA, 1985; Brown and Kaplan, 1987; Blair et al.,

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[3]The NTP (1986) study of perchloroethylene received peer review by the NTP Board of Scientific Counselors. The panel of experts agreed (nine affirmative votes) that there was clear evidence of carcinogenicity of perchloroethylene for B6C3F1 mice as shown by increased incidences of both hepatocellular adenomas and carcinomas in males and of hepatocellular carcinomas in females. The panel approved (eight affirmative votes, one negative vote) conclusions of some evidence in female F 344/N rats of carcinogenicity of perchloroethylene as shown by increased incidences of mononuclear cell leukemia. The panel concluded (five affirmative votes, four negative votes) that there was clear evidence of carcinogenicity of perchloroethylene in male F344/N rats as shown by an increased incidence of mononuclear cell leukemia and uncommon renal tubular cell neoplasms. (Two members of the expert panel abstained from voting due to employment conflict of interest.)

[4]There is agreement between IARC and EPA that the animal evidence is closer to "sufficient" than to "limited." IARC has classified perchloroethylene in the IARC category that is more similar to EPA's sufficient animal evidence, B2. The IARC phraseology, however, is "possible" human carcinogen, whereas EPA uses the term "probable" human carcinogen.

1990). Laboratory data published subsequent to the perchloroethylene carcinogenicity bioassays have led to the development of new hypotheses about the mechanisms of perchloroethylene tumorigenesis, however, and reasoning, discussed in this paper, has been put forward suggesting species specificity for certain metabolic pathways as well as the proposed tumorigenesis mechanisms involving peroxisomes in the livers of mice and hyaline droplets in male rat kidneys. Such hypotheses imply that certain experimental results from the animal carcinogenicity bioassays are of questionable predictive validity with respect to human health hazards, and so the question now must be answered whether the general assumption of "sufficient" animal data indicating a human hazard potential remains a reasonable assumption for perchloroethylene.

Implications of Perchloroethylene Metabolism

It is generally considered that the toxicity, mutagenicity, and carcinogenicity of perchloroethylene resides in reactive metabolites. The data do not lend support to classifying the parent compound per se as a mutagen, although certain perchloroethylene metabolites may be mutagenic (e.g., chloroacetaldehydes including chloral hydrate). Several perchloroethylene metabolites have been shown to be cytotoxic, and certain metabolites (e.g., TCA, DCA, and trichloroacetaldehyde), cause liver tumors in mice.

Studies in both animals and humans indicate that metabolism of perchloroethylene is relatively limited as evidenced by the fact that a high percent of absorbed dose is excreted in the breath as the parent molecule. In human studies, however, only approximately half of the perchloroethylene absorbed has been accounted for through the excretion of parent compound or its metabolites.

There is no reason to believe that qualitative differences exist between species with respect to oxidative metabolism of perchloroethylene, although there are quantitative differences. For example, metabolic rates differ among species, saturation of metabolism pathways occurs at different levels in different species, and other parameters such as peak blood levels and half times of the parent compound and its metabolites may vary among species. Thus, higher blood levels of TCA, for example, may be reached in mice, but TCA production and half life is much longer in humans. Metabolites from oxidative pathways

probably contribute to the development of liver tumors in mice. Several perchloroethylene oxidative metabolites may be potentially involved in different mechanisms hypothesized for mouse hepatocarcinogenicity.

Although certain metabolites of oxidative metabolism may be mutagenic (e.g., the chloroacetaldehydes), these positive data are predominantly limited to in vitro studies. Moreover, perchloroethylene was assayed in the presence of several types of metabolic activation systems (e.g., liver homogenates and intact hepatocytes) that would favor oxidative metabolism and under these conditions predominantly negative results were found.

Perchloroethylene may also be activated by a minor, but possibly important, secondary pathway involving hepatic conjugation with glutathione followed by renal processing of the S-conjugate. This S-conjugate is a potent beta-lyase dependent mutagen in the *Salmonella*/mammalian microsome assay. Mutagenic metabolites formed in the kidney would be consistent with the tumors observed in male rat kidneys. However, these mutagenicity studies are in vitro and have been conducted in only one laboratory. The beta-lyase pathway activation of the S-cysteine conjugate may lead to metabolites that are also nephrotoxic.

Glutathione conjugation of perchloroethylene by human liver has not been shown, although only a very few human liver samples have been examined, and even the enzyme activity observed in rodents was low. The quantitative relationships between various degrees of saturation of the oxidative pathway and concomitant enhancement of the conjugative pathway requires close scrutiny before it can be concluded that conjugation is not important in perchloroethylene metabolism by humans.

The available data indicate that metabolism would be a prerequisite for perchloroethylene cytotoxicity and mutagenicity. Several studies are now available on the mutagenicity of certain perchloroethylene metabolites, and their results warrant further consideration. The mutagenicity studies on metabolites of perchloroethylene emphasize the need for additional studies concerning the mutagenic role of intermediates in perchloroethylene carcinogenesis. Other pathways such as the further metabolism of TCA, or other routes to CO2 initially proposed in the 1985 HAD, or as yet undiscovered metabolic pathways may be important.

Mouse Liver Tumors

At this time, the Agency's position is that mouse liver tumors are considered evidence for human carcinogenic potential, although the evidence may be downgraded on a case-by-case basis according to chemical-specific data. The current EPA policy for evaluating mouse liver tumor data is described in the guidelines for carcinogen risk assessment, published in 1986 (U.S. EPA, 1986).

Many different mechanisms have been proposed for hepatocarcinogenicity in B6C3F1 mice. One of the mechanisms hypothesized for perchloroethylene liver tumor induction in these mice is postulated to result from TCA-induced peroxisome proliferation, and is said to be species-specific. There is some evidence to support the hypothesis that perchloroethylene-induced hepatic carcinogenesis may be related to peroxisome proliferation; critical review of the scientific literature, however, reveals significant data gaps regarding the relationship between the proliferative effect and neoplasia.

For example, if peroxisome proliferation is causally related to the induction of liver cancer, one would expect to detect a quantitative relationship between the two events. That is, potent peroxisome proliferators should also be potent hepatocarcinogens. This does not appear to be the case. Also, if mouse liver peroxisome proliferation in response to perchloroethylene is correlated to the induction of liver cancer, strains of mice that exhibit peroxisome proliferation should also develop liver tumors. Trichloroethylene, a structurally-related hepatocarcinogen in B6C3F1 mice, and its metabolite TCA, induce peroxisome proliferation in Swiss mice, but trichloroethylene does not cause liver tumors in this strain.

The results of recent studies have raised the possibility that genotoxicity may occur independently of peroxisomal proliferation following exposure to perchloroethylene. Other evidence indicates mutagenicity of metabolites should be further considered.

The role of the c-myc and C-H-ras oncogenes in the development of liver tumors in B6C3F1 mice needs further evaluation. Nelson et al. (1990) reported that TCA administration significantly increased expression of the c-myc oncogene in hepatocellular carcinomas in B6C3F1 mice. These workers suggest that TCA increased c-myc expression may prevent initiated cells from differentiating, thereby increasing their probability of progressing to hepatocellular carcinoma.

Thus, although evidence does exist that supports the hypothesis of mouse liver cancer induction being secondary to peroxisome proliferation, the validity of the hypothesis remains questionable. It is clear that perchloroethylene, probably through its metabolites, causes peroxisome proliferation and also causes hepatocellular carcinoma in B6C3F1 mice, but a cause-and-effect relationship between the two effects has not been shown. It is not clear what role, if any, peroxisome proliferation actually plays in perchloroethylene carcinogenesis. Additional studies of the mouse peroxisome- proliferator-activated receptor, a member of the steroid hormone receptor superfamily, may contribute to understanding any role the peroxisome proliferation phenomenon might have in tumorigenesis.

Also, several other proposed mechanisms need to be further investigated. For example, the recent demonstration that the major metabolite of perchloroethylene, TCA, causes the expression of the c-myc oncogene in B6C3F1 mice requires experimental exploration. Possible roles of other toxic metabolites, including mutagenicity, need further consideration as well.

Kidney Tumors in Male Rats

Since the NTP discovery that chronic administration of perchloroethylene induces a low level of renal tubule cell tumors in male rats, significant research has been conducted to explain the mechanism of the carcinogenic effect. This research has resulted in at least three possible explanations.

The tumors may be secondary to the renal accumulation of the low molecular weight protein, alpha-2μ-globulin. Since only male rats produce the protein, if this is the mode of tumor production, the tumors may have no predictive validity with respect to human health hazard on a site- or mechanism-specific basis.

Hyaline droplet accumulation in rat kidneys has not been demonstrated to occur, however, at the perchloroethylene inhalation doses that caused renal tumors in male rats. The alpha-2μ-globulin response for perchloroethylene is relatively mild, and the pathology reported is not entirely consistent with the results generally observed for chemicals that cause alpha-2μ-globulin accumulation. Renal hyperplasia was observed in a female rat and kidney adenocarcinoma was identified in a male mouse. In the case of perchloroethylene it is

feasible, however, that the renal tumors could result in part from the accumulation of the alpha-2μ protein. Enhanced cell replication, associated with hyaline droplet formation, and mutagenic metabolites from the beta-lyase pathway may together lead to the observed renal tumors. The role of alpha-2μ-globulin in perchloroethylene-induced rat kidney tumorigenesis is not understood; thus, the tumors cannot be discounted. The EPA is presently developing criteria which will define a weight-of-evidence approach for evaluating, on a case by case basis, the role of alpha-2μ-globulin in rat kidney tumor formation.

The chronic administration of perchloroethylene produces nephrotoxicity and it has been suggested that tumor production is secondary to sustained cytotoxicity and cellular regeneration. If so, it is probably related to alpha-2μ-globulin nephropathy, although sustained chronic nephrotoxicity independent of alpha-2μ-globulin accumulation has been implicated as a possible mechanism of perchloroethylene-induced renal carcinogenesis in male rats. Perchloroethylene-induced renal tubule neoplasms have been detected only in male rats, but certain nontumor pathology associated with the chronic administration of perchloroethylene has been detected in female rats and in both sexes of mice, as well as in male rats. These chronically-induced perchloroethylene nonneoplastic kidney lesions exhibit neither species- nor sex-specificity. Such a mechanism implies that kidney tumors would not occur solely in male rats. If tubule cell neoplasia were directly consequent to this pathology, tumors would likely have been found in dosed female rats or male or female mice. Although nephrotoxicity may play a role, more supportive evidence is needed to define what that role may be. The fact that there is little doubt that the kidney is a target organ for perchloroethylene and other chlorinated ethanes and ethylenes in mammalian species contributes to the overall concern regarding the kidney tumor end point.

A glutathione-beta lyase conjugation pathway of perchloroethylene metabolism has been discovered in rodents. This minor pathway leads to the formation of a cytotoxic/mutagenic metabolite(s). Humans may or may not have the capacity to carry out the initial conjugation reaction. If the human cannot form the perchloroethylene-glutathione conjugate, this pathway is irrelevant with respect to hazard projection.

OHEA is unable at this time to conclude that results with human liver samples are indicative of a general inability of humans to conjugate perchloroethylene. This is because

such few liver samples were studied, and a conjugation rate tenfold lower than that observed for rats would fall below the limits of detection of the method employed. Tenfold differences in enzyme activities within the human population are not uncommon.

While there is some evidence to support each of the proposed mechanisms, there are also significant quantitative and qualitative gaps in the supportive data. The mode of perchloroethylene-tumorigenesis in male rats is not yet understood.

Leukemia in Rats

Under the conditions of the NTP study there was evidence of a carcinogenic effect of perchloroethylene in male and female rats as evidenced by significant increases in the incidences of MCL in both sexes. However, the usefulness of increased incidences of MCL in the prediction of human carcinogenic risk associated with exposure to perchloroethylene has been questioned on several grounds: high spontaneous background incidences, use of special supplemental analyses to aid in data interpretation, and the relevance of MCL in F344/N rats because this type of leukemia does not occur in humans.

The leukemia incidences were statistically significantly increased in both male and female rats. In both sexes, perchloroethylene caused a dose-related increase in severity of MCL and shortened the time to tumor in female rats. There was an increased incidence of advanced MCL in female rats that died before the scheduled termination of the study. The supplemental analyses were based on standard methods of data evaluation and lent support to the data interpretation.

While human leukemias originate in bone marrow, MCL is splenic in origin. Despite the fact that the disease occurs only in rats, it is a neoplasm whose incidence and progression can be influenced by chemical agents. Discounting a rodent neoplasm simply because it has no exact human counterpart is not reasonable. Many aromatic amines are probable bladder carcinogens in humans but are likely to produce Zymbal gland tumors rather than bladder tumors in rats. The human does not have a Zymbal gland.

There is no one specific mechanism that explains all of the tumor end points. Of several modes of action that have been proposed for perchloroethylene tumorigenesis, different mechanisms have been hypothesized to cause the three distinct tumor types

observed in rats and mice. Data exist to support certain of these mechanisms. Scientific evidence also exist to support species specificity for particular mechanisms, but the incompleteness of the data limits the scope of the conclusions that can be deduced although the mechanisms are certainly plausible. The modes of action for perchloroethylene carcinogenesis are not yet well understood. With regard to the kidney tumors, evidence exists for more than a single mechanism, all of which may play some role in the tumor development independently or in concert. A cause and effect relationship cannot be effectively demonstrated between the peroxisome proliferation and development of liver tumors in mice. Several other mechanisms may contribute to hepatocarcinogenicity in mice. The possibility that there may well be a mutagenic component in the development of the tumors, especially the kidney tumors, cannot be entirely ruled out. The evidence that metabolic pathways in rodents may not occur in humans is not convincing.

Because mechanisms of perchloroethylene carcinogenesis are not well enough understood, each individual tumor type is viewed as contributing not just to the "sufficient" evidence in animals, but to the overall weight-of-evidence determination that perchloroethylene is a probable human carcinogen. In each case, reasonable doubt exists that the mode of tumorigenesis is only through mechanisms species-specific to rodent strains. Other mechanisms are feasible that would not be specific to rodents. All three tumor types can therefore be considered valid as indicators relevant to potential carcinogenicity in humans, although some uncertainty does exist concerning relevance to humans.

EPA's Guidelines for Carcinogen Risk Assessment (EPA, 1986) suggest that the weight of evidence increases with the increase in number of animal species, strains, sexes, and number of experiments and doses showing a carcinogenic response, with the increase in number of tissue sites affected , with the occurrence of dose-response relationships as well as statistical significance of the increased tumor incidence in treated compared to control groups, when there is decreased time-to-tumor occurrence or death with tumor, and when there is a dose-related increase of malignant tumors. All of these criteria are met in some way. Perchloroethylene causes at least three types of tumors in rodents, each of which can be considered as contributing in some way to the concern for cancer-causing potential in humans. Indications of cancer-causing activity were seen in two species, in two sexes, by

inhalation and oral exposure, and is called "sufficient" animal evidence; and, although there is some scientific uncertainty concerning relevance to humans for some of the data, the totality of the animal data for perchloroethylene is not only closer to the "sufficient" evidence category, but also can be considered relevant for extrapolation of hazard potential to humans.

Therefore, although the relevance of some of the data is less than certain, the inclusive animal data for perchloroethylene, taken as a whole, along with the considerations of inadequate human data, information on metabolism, and mutagenicity data on metabolites are most logically categorized in Group B2, thus classifying perchloroethylene as a probable human carcinogen. It must be remembered that classifications refer only to the weight of the experimental evidence that a chemical is carcinogenic and not to its potency of carcinogenic action. This paper has not addressed the quantitative estimation of risk. Mechanistic considerations may justify special interpretation of the dose-response data with respect to projecting human carcinogenesis risk.

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