



**SCIENTIFIC REVIEW PANEL VERSION
TECHNICAL SUPPORT DOCUMENT**

PART C

PROPOSED IDENTIFICATION OF

PERCHLOROETHYLENE

AS A TOXIC AIR CONTAMINANT

MAY 1991

**State of California
Air Resources Board**

This report has been reviewed and approved by the staffs of the California Air Resources Board and the Department of Health Services. The contents do not necessarily reflect the views and policies of the Air Resources Board or the Department of Health Services, nor does mention of trade names of commercial products constitute endorsement or recommendation for use.

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PART C

STAFF RESPONSES TO PUBLIC COMMENTS ON THE
PERCHLOROETHYLENE TECHNICAL SUPPORT DOCUMENT

Prepared by the Staffs of the Air Resources Board
and the Department of Health Services

May 1991

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PART C
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I. COMMENT LETTERS RECEIVED

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2/3/90

Mr. Donald J. Ames, Assistant Chief
Stationary Source Division
Air Resources Board
Attn: Perchloroethylene
PO Box 2815
Sacramento, CA 95812

FAX 916/322-6003



Dear Mr. Ames,

The following are our comments on the Draft Report on Perchloroethylene Part A, of December 1989.

Additional sources of indoor exposure must be assessed

There has been a failure in Part A of the report to adequately assess the total public exposure to Perchloroethylene (PCE) from all indoor environments as required by state statute. The report only assesses exposure from indoor air in the home, and neglects to consider exposure in such places as hospitals, schools, offices, and the work place. For instance, an employee at a dry-cleaner can face exposure at work and in the home to potentially high daily levels of PCE, and needs to be considered. Exposure to a consumer at a dry cleaning counter should also be assessed. Information must be added to the final report to adequately assess the multiple routes of exposure from the different sources of PCE in indoor air.

California Health and Safety Code Article 3, Section 39950.5 (a) states that "In evaluating the level of potential human exposure to toxic air contaminants, the state board shall assess that exposure in indoor environments as well as ambient air conditions."

(c) "When the state board identifies toxic air pollutants that have been found in any indoor environment, the state board shall refer all available data on that exposure and the suspected source of the pollutant to the state Department of Health Services..." (emphasis added)

(d) "In assessing human exposure to toxic air contaminants in indoor environments pursuant to this section, the state board shall identify the relative contribution to total exposure to the contaminant from indoor concentrations, taking into account both ambient and indoor air environments." (emphasis added)

(cont)

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Need further data on PCE in food supply

The estimation of daily intake from ingestion of PCE through food represents a potentially high risk based on the cited European data. In order to assess the relevance of this data to the California consumer, information on the mechanisms by which PCE contaminates food supplies should be presented. If PCE in the food supply is mainly present due to adsorption onto fats in foods through contact with airborne PCE, then data comparing concentrations of airborne PCE in California and Europe could help establish whether the European data could be representative of California also. Again, in addition to concentrations and daily intake figures, associated cancer risk figures should be presented related to ingestion.

Include worst case numbers for assessment of exposure

For studies of indoor air concentrations of PCE, only 24-hour averages were given. Maximum concentrations should also be included so that the worst case exposure can be assessed. Information on individual cancer risk associated with worst case and average concentrations should also be included.

In addition, maximum daily and maximum chronic exposure for people using several consumer products containing PCE, who also use dry-cleaners frequently, who work in environments with high levels of PCE, who live in areas with high background levels in ambient air and/or live in PCE hotspot areas near high emissions sources, who ingest high levels through food or water supplies, and who are exposed to any other source of PCE, should be considered, and total worst case daily intake and cancer risk data should be presented.

Append to report data from federal SARA III TRI database

It is highly appropriate for the purposes of the Toxic Air Contaminant (TAC) identification process to include in the report information on specific sources of PCE emissions. The final report should append the 1987, 1988, and 1989 data identifying PCE emissions from all sources in the federal SARA Title III database, which are readily available. The public has the right to know of and review such data, and the ARB TAC documents should provide this information on a geographic basis.

(cont)

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FAX 916/322-6003



Add cancer risk and emissions data for hotspot sites assessed

- For the South Coast sites assessed for PCE emissions (p.A-33), there was no inclusion of information on cancer burden but only on concentrations. Individual and population cancer risk should be included in the final report. In addition, no information was given on the emissions from these sites, which would be useful for comparison purposes. This information should be included in the final report.

Thank you for your consideration. We will be reviewing and commenting on the final report also.

Sincerely,

Julia E. May
Research Associate

A large, fluid handwritten signature in dark ink, which appears to read "Julia E. May". The signature is written in a cursive style with a long, sweeping underline that extends to the right.



HALOGENATED SOLVENTS INDUSTRY ALLIANCE

1225 19th Street, N.W. Suite 300, Washington, D.C. 20036-2411 • (202) 223-5890

February 1, 1990

Mr. Donald O. Ames
Assistant Chief
Stationary Source Division
Air Resources Board
Attn: Perchloroethylene
P. O. Box 2815
Sacramento, CA 95812

Stationary Source
Division
Air Resources Board

Dear Mr. Ames:

The Halogenated Solvents Industry Alliance (HSIA) offers the enclosed comments on the draft Technical Support Document: Proposed Identification of Perchloroethylene as a Toxic Air Contaminant (Part B: Health Effects of Perchloroethylene).

HSIA is an association of producers, distributors, importers, and users of halogenated solvents, including perchloroethylene. Our members, as well as other users of perchloroethylene have a vital interest in the accuracy and scientific validity of the Report.

Sincerely,

Paul A. Cammer

Paul A. Cammer, Ph.D.
President

Enclosure

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BEFORE THE CALIFORNIA AIR
RESOURCES BOARD

COMMENTS OF THE
HALOGENATED SOLVENTS INDUSTRY ALLIANCE
ON THE
DRAFT TECHNICAL SUPPORT DOCUMENT:
PROPOSED IDENTIFICATION OF PERCHLOROETHYLENE
AS A TOXIC AIR CONTAMINANT
(Part B: Health Effects of Perchloroethylene)

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February 1, 1990

Paul A. Cammer, Ph.D.
President

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BEFORE THE
CALIFORNIA AIR RESOURCES BOARD

COMMENTS OF THE
HALOGENATED SOLVENTS INDUSTRY ALLIANCE
ON THE
DRAFT TECHNICAL SUPPORT DOCUMENT:
PROPOSED IDENTIFICATION OF PERCHLOROETHYLENE
AS A TOXIC AIR CONTAMINANT
(Part B: Health Effects of Perchloroethylene)

Introduction

The Halogenated Solvents Industry Alliance (HSIA) offers these comments to the Air Resource Board (ARB) on Part B (Health Effects of Perchloroethylene) of the draft Technical Support Document for the Proposed Identification of Perchloroethylene as a Toxic Air Contaminant. Our comments include a summary of the

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pertinent literature on the carcinogenic potential of perchloroethylene in animals and humans, a discussion of the importance of species differences in metabolism of this chemical, and a recommendation to develop a more plausible estimate of potential risk that incorporates pharmacokinetic information.

HSIA is an association of users, distributors, and producers of chlorinated solvents, including perchloroethylene. Our members, as well as other users of perchloroethylene, have a vital interest in the accuracy and scientific validity of the Technical Support Documents which serve as the basis for the proposal to identify perchloroethylene as a toxic air contaminant. Decisions made by the ARB on the basis of the Technical Support Documents will have a significant effect on actions taken by local air districts in California to regulate perchloroethylene, on possible future proposals from Cal-OSHA to change worker exposure levels, and on other risk assessment-related legislation and regulation within the state. As a consequence of those actions, a large number of industrial and commercial users of perchloroethylene will be affected, as will the public that benefits from the applications of the chemical.

Executive Summary

The overall weight of the scientific evidence for perchloroethylene suggests that it is unlikely to pose a carcinogenic risk to humans at ambient environmental or occupational exposure levels. The health effects of perchloroethylene have been studied extensively.

Long-term bioassays of perchloroethylene have shown that it produces liver cancer in certain species of mice, but not in rats. The proximal carcinogen appears to be trichloroacetic acid, a metabolite of perchloroethylene, which induces proliferation of peroxisomes in liver cells. Humans produce less trichloroacetic acid than mice and rats, and do not exhibit the critical biological response of peroxisome proliferation, which is responsible for the formation of liver tumors in rodents. These documented species differences in response to perchloroethylene exposure between mice and rats, and between rodents and humans, provide strong support for the conclusion that the chemical does not pose a carcinogenic risk to humans.

Adding support to this conclusion are the findings of epidemiologic studies in workers exposed to perchloroethylene. The overall results of epidemiology studies carried out on drycleaner workers to date do not provide support for the conclusion that perchloroethylene poses a cancer risk to humans.

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The draft report must be revised to reflect more clearly the significant qualitative differences in metabolism of perchloroethylene between mice and rats, and between rodents and humans, and the negative evidence for carcinogenicity in human studies. The weight of the available scientific evidence, as reviewed by the International Agency for Research on Cancer (IARC) and the Science Advisory Board of the U.S. Environmental Protection Agency (EPA), does not support the conclusion that the chemical is a probable human carcinogen.

If the final report includes an estimate of potential risk, HSIA strongly recommends that it incorporate available pharmacokinetic information. In the past year, both ARB and its Scientific Review Panel (SRP) have recommended that the Department of Health Services go further to incorporate pharmacokinetic information into its risk assessments. Although the pharmacokinetic data for perchloroethylene are discussed in the draft report, the final risk assessment range ($5-21 \times 10^{-6}$ for a 1 ug/m^3 lifetime exposure) was calculated without incorporating pharmacokinetic information. Since this is the range that will be used for regulatory purposes, pharmacokinetic information has for all practical purposes not been reflected. This is flatly inconsistent with the California Carcinogen Guidelines ("Guidelines") which provide (A-16) that "[p]harmacokinetic data on metabolism of dosed substances,

effective dose at target site, or species differences between laboratory test animals and humans should be considered in dose-response assessments when they are available."

The DHS risk estimate is more than an order of magnitude higher than the most recent range under consideration by EPA ($2.9-9.5 \times 10^{-7}$), which was calculated using pharmacokinetic data. Incorporating metabolic and pharmacokinetic data into the risk calculations will reduce the uncertainties inherent in the risk assessment process and will result in a more plausible estimate of risk to humans. In light of the availability of extensive pharmacokinetic data (as reflected in the draft) and the Guidelines requirement that they be taken into account, rejection of a physiologically-based pharmacokinetic model in favor of an approach based on default assumptions appears to be an abuse of the Agency's discretion.

Carcinogenic Potential of Perchloroethylene

I. Experimental Animal Data

The principal animal carcinogenicity studies reviewed in the draft are summarized below.

A. 1978 Rampy Study

In this inhalation study, groups of male and female Sprague-

Dawley rats were exposed to 0, 300, and 600 ppm of the chemical for 6 hours per day, 5 days per week for 12 months. They were then observed for an additional 18 months. No increase in tumors in the exposed animals was observed.

B. NCI Bioassay

An increased incidence of mouse liver tumors was observed in a National Cancer Institute (NCI) gavage bioassay reported in 1977. There are a number of controversies surrounding the use of this study to determine human cancer risk. The National Research Council, in a review of the NCI study, stated that "the quantities of TCE [tetrachloroethylene] given were so large that marked, dose-dependent mortality in both species occurred throughout the study period" (NRC, 1980). It concluded that "the findings of this study should be interpreted with caution, recognizing the limitations of the experimental design (e.g., massive doses of TCE, large volumes of oil vehicle, marked nephrotoxicity, diminished lifespan)." We question whether this is "properly conducted bioassay," as required by the Guidelines, for the evaluation of potential human hazard from perchloroethylene.

C. NTP Bioassay

The National Toxicology Program (NTP, 1986a) conducted an inhalation bioassay and concluded that the study showed clear evidence of carcinogenicity in male Fischer 344 rats, based on an increase in the spontaneous incidence of mononuclear cell leukemias and a non-statistically significant increase in renal adenomas and carcinomas; some evidence of carcinogenicity in female rats based on an increase in the spontaneous incidence of mononuclear cell leukemias; and clear evidence of carcinogenicity in male and female B₆C₃F₁ mice based on an increase in the spontaneous incidence of hepatocellular adenomas and carcinomas.

II. Interpretation and Significance of Animal Bioassay

Results

A. Rat Mononuclear Cell Leukemia

EPA's Science Advisory Board determined that the high spontaneous incidence of rat leukemias observed in the NTP inhalation bioassay was not related to perchloroethylene exposure. Slight increases in mononuclear cell leukemia, which occurs spontaneously at a high and variable incidence in the Fischer 344 rat, were observed in male and female rats exposed to 200 or 400 ppm tetrachloroethylene for two years. Incidences increased from 36 percent in control male rats to approximately

60 percent in exposed males and from a control value of 56 percent to approximately 80 percent in exposed female rats. Importantly, the incidence of mononuclear cell leukemia in the exposed rats was not dose-related. The increased incidence observed is unconvincing with respect to human risk since (i) questions were raised involving whether the proper staging criteria were used, (ii) it is uncertain whether there is actually a human counterpart for this type of leukemia, and (iii) the control incidences exceed those previously seen in the testing laboratory, and also exceed the NTP historical control value. In this regard, it should be noted that incidence rates in the tetrachloroethylene-treated rats were 37/50 (male 200 ppm), 37/50 (male 400 ppm), 37/50 (female 200 ppm), and 29/50 (female 400 ppm). Comparison to a study conducted at the same laboratory at the same time shows leukemia rates in untreated rats of 34/50, (NTP, 1986b).

The etiology and pathogenesis of mononuclear cell leukemia in the rat are unclear, which has to this point precluded mechanistic study of this response. Be that as it may, several associations in the response render the biological significance of this observation for humans doubtful. As noted above, it is exceedingly common in the Fischer 344 rat. Importantly, leukemia was not observed in the 1977 NCI study (Osborne-Mendel rats) nor by Rampy (Sprague-Dawley rats) in the study described above. Nor has tetrachloroethylene induced leukemia in mice. These data, coupled with the apparent lack of genotoxicity of

tetrachloroethylene, lead us to conclude that the observed Fischer 344 rat mononuclear cell leukemia is a strain-specific phenomenon and, as the Science Advisory Board stated, is not of relevance to humans.

B. Male Rat Kidney Tumors

As to the other result in rats, a marginal, non-statistically significant increase in kidney tumors observed in the same study, the Board stated that the mechanism responsible "appears to be unique to male rats", and that recent research "indicates that for many halogenated organics, probably including perchloroethylene, the mechanism producing these types of tumors is probably not operative in humans and, therefore, may not be relevant for human risk assessment" (SAB, 1988).

Two renal tubular cell adenocarcinomas were observed in male rats in the NTP study at 400 ppm, while none were observed at 200 ppm or in the control group. Two renal tubular cell adenomas were also observed in the 400 ppm group of male rats versus three at 200 ppm and one in the control group (NTP, 1986a). This sex-specific observation is not without precedence -- small increases in male rat kidney tumors have been occasionally noted across the class of short-chain aliphatic hydrocarbons. Renal tubular cell neoplasms have been shown to be produced by unleaded gasoline in male rats only.

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An elegant series of experiments conducted on unleaded gasoline at the Chemical Industry Institute of Toxicology (CIIT) has shown the male rat kidney tumor response to be related to the following events: complexing of trimethylpentane with an alpha-2u-globulin, renal tubular absorption of the complex, renal tubular hyaline droplet formation, renal tubular injury, enhanced renal tubular regeneration, and subsequent tumor development secondary to ongoing injury. Perchloroethylene, under the conditions of the NTP study, has been shown to be nephrotoxic and has been shown, like gasoline, also to elicit this hyaline droplet nephropathy in exposed male, but not female, rats. In recent experiments conducted at CIIT (Goldsworthy et al., 1988) and at the Central Toxicology Laboratory at ICI, Ltd., oral gavage administration of 1000 mg/kg and inhalation of 1000 ppm perchloroethylene 6 hours/day for 10 days resulted in hyaline droplet formation in the P2 segment of the proximal tubule. The work at CIIT has further confirmed deposition of alpha-2u-globulin in renal proximal tubule cells, resulting in cellular toxicity, and an increase in cellular regeneration in the P2 segment of the proximal tubule. Thus, perchloroethylene, in addition to its generalized nephrotoxicity at doses used in the NTP study, has been shown to elicit the male rat-specific hyaline droplet nephropathy. This is a qualitatively different response (male-rat specific) which would not be expected to occur in humans.

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It should be mentioned that data from the laboratory of Dr. Henschler in Germany suggest the existence of a second, very minor metabolic pathway involving perchloroethylene conjugation with glutathione (Dekant et al., 1986). This conjugate represents approximately 0.1 percent of the administered dose in the mouse and approximately 0.01 percent in the rat based on pooled 72-hour urine. Dr. Henschler has suggested that this minor metabolite may be a substrate for renal beta-lyase, producing an electrophilic metabolite that could potentially react with renal tissue macromolecules including DNA. The existence of a perchloroethylene-glutathione conjugate in mice and rats has been identified at ICI. The minor metabolic pathway, GSH-transferase, has been shown to be approximately six times more active in rats than in mice, and renal, beta-lyase activity to be approximately five to six times higher in rats than in mice. Within its limits of detection, the ICI laboratory has found no evidence for conjugation of perchloroethylene with GSH in human tissue.

Thus, while this minor pathway could conceivably have played a role, in concert with renal cytotoxicity as well as male rat-specific hyaline droplet formation, humans appear qualitatively different from rats due to the apparent absence of formation of the initial GSH conjugate. Moreover, the absence of a direct conjugate-mediated genotoxic effect was demonstrated in

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rats at CIIT by the failure of perchloroethylene to induce DNA repair (UDS) in the rat kidney using in vivo/in vitro techniques (Goldsworthy et al., 1988).

C. Mouse Liver Tumors

Some scientists believe that liver tumor findings in the B₆C₃F₁ mouse, in the absence of demonstrable direct genotoxic activity, but in the presence of other epigenetic (promotional) events, are not relevant to humans. The high spontaneous incidence of these tumors complicates both the statistical and biological evaluation of the weight of the evidence. Two recent papers show that DNA from liver tumor tissue taken from untreated B₆C₃F₁ mice that have spontaneously developed the tumors expressed the active H-ras oncogene (Fox and Watanabe, 1985; Fox et al., 1987). These studies indicate that the B₆C₃F₁ mouse may be genetically predisposed to liver tumors, making it an inappropriate model for direct comparison to the human.

Perchloroethylene, in common with many nongenotoxic promoters of carcinogenesis, induces hepatic peroxisome proliferation and cellular replication in B₆C₃F₁ mice (but not rats) at levels used in chronic bioassays. Moolgavkar describes how a multi-stage process consisting of initiation (i.e., by a direct genotoxic agent; by indirect effects on the genome secondary to the pharmacologic/toxicologic effects of an agent; or by oncogene

activation via a spontaneous or chemically induced mechanism) followed by cellular replication induced by a nongenotoxic agent would lead to an increased incidence of tumors (Moolgavkar and Knudson, 1981). This theoretical explanation suggests that perchloroethylene, a nongenotoxic agent, would be markedly less likely to induce tumors in species that are not genetically predisposed.

In sum, there is widespread scientific agreement that an increased incidence of mouse liver tumors, in the absence of other significant carcinogenic effects, is of questionable significance in assessing cancer risk to humans (International Expert Advisory Committee to the Nutrition Foundation, 1983; Schach von Wittenau and Estes, 1983; Butler and Newberne, 1975; Tomatis et al., 1973; Grasso and Crampton, 1972). One reason is the very high spontaneous incidence of liver tumors in mice. The recent identification of an oncogene in B₆C₃F₁ mouse liver tumors casts further doubt on the value of mouse liver tumors as an end-point in assessing human risk.

III. Species Differences

The increased incidence of liver cancer in mice exposed to perchloroethylene appears to have resulted from peroxisome proliferation, a mechanism to which rodents are extremely sensitive relative to primates and humans. In addition, perchloroethylene is biotransformed to trichloroacetic acid, the

proximat peroxisom -prolif rating agent, to a greater extent in mice than in rats. Humans, in turn, bioactivate even less perchloroethylene than do rats.

Trichloroacetic acid has itself been shown to cause peroxisome profileration in mouse liver cells and to induce liver tumors in mice when given alone (Herren-Freund et al., 1986). The level of peroxisome proliferatation in perchloroethylene-exposed mice corresponds closely to the level of trichloroacetic acid production (Odum et al., 1988). Significantly, research has shown that peroxisome proliferatation does not occur in human liver cells following in vitro exposure to trichloroacetic acid (Elcombe, 1985).

Specifically, mechanistic studies published by scientists at CIIT and ICI's Central Toxicology Laboratory have shown peroxisome proliferatation to be induced in mice, but not rats, after repeated gavage dosing of 1000 mg perchloroethylene/kg/day for 10 days, and after inhalation of 200 or 400 ppm perchloroethylene for up to 28 days (Odum et al., 1988; Goldsworthy and Popp, 1987). The induction of peroxisomes has been shown to be directly related to the metabolism of perchloroethylene to trichloroacetic acid. After exposure to 400 ppm perchloroethylene for 6 hours, peak blood levels of trichloroacetic acid were 13 times greater, and the area under the plasma concentration vs. time curve 6.7 times greater, in mice than in rats. Statistically significant increases in the peroxisomal

marker nzyme -- CN insensitive palmitoyl CoA activity -- as well as increased numbers of hepatic peroxisomes, were also observed in the mice. Consistent with the marked relative insensitivity of higher mammalian species, including humans, to peroxisome proliferating agents, trichloroacetic acid did not stimulate peroxisome enzymes in human cells in culture.

These data strongly suggest that perchloroethylene would not cause liver tumors in humans (Stott, 1988). Even if peroxisome proliferation is only a marker of liver cell involvement in the carcinogen process, it is clear that human cells have a qualitatively different reaction to the rodent proximal carcinogen. Thus, humans are doubly unlikely to show a carcinogenic response to perchloroethylene due to (i) significantly lower production of trichloroacetic acid, and (ii) the relative insensitivity of humans to peroxisome proliferation, the critical biochemical response. Indeed, the SAB has indicated that a mechanistic model such as peroxisome proliferation is likely to be important for perchloroethylene (SAB, 1988).

Induction of peroxisomes is not thought to be a linear function of trichloroacetic acid concentration in the liver and, in the case of perchloroethylene, is accomplished by the induction of DNA synthesis in the $B_6C_3F_1$ mouse liver. The data suggest that peroxisome proliferation and induction of hepatic DNA synthesis, along with the genetic predisposition of the $B_6C_3F_1$ mouse liver to liver tumor induction, act in

concert to enhance tumor formation in perchloroethylen -exposed $B_6C_3F_1$ mice. By analogy to trichloroethylene, it appears that genetic predisposition is a critical factor. Henschler has reported that, after oral exposure to purified, amine-stabilized trichloroethylene, Swiss mice do not appear to develop hepatic tumors (Henschler et al., 1984). $B_6C_3F_1$ mice do develop hepatic tumors, on the other hand, although both strains are thought to metabolize trichloroethylene in a similar fashion quantitatively.

The foregoing data, taken together, overwhelmingly support the concept that perchloroethylene appears to be enhancing spontaneous liver tumors in $B_6C_3F_1$ mice by a secondary (promotional) mechanism, which conceptually embodies the principle of a practical threshold. If a substance acts as a promoter, rather than an initiator, the dose-response relationship would be expected to exhibit a practical threshold (Ames et al., 1987). Much of the scientific research referenced above is neither discussed nor referenced in the draft report. Copies of the relevant articles are attached. It is very important that the final report take into account these recent scientific findings showing a clear species difference in response to perchloroethylene. In this regard, we would be pleased to arrange for a meeting with the Air Resources Board or Department of Health Services staff to discuss this scientific information.

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IV. Epidemiology

Overall, the draft report's evaluation of the epidemiology data base is consistent with that of EPA's Science Advisory Board (SAB) which stated in 1985 that the data available from the six epidemiology studies on dry cleaning workers exposed to perchloroethylene and other solvents as of the time of the Board's review "do not substantiate the hypothesis that the chemical is carcinogenic to humans" (SAB), 1985). Interpretation of the majority of these studies is complicated by the existence of one or more of the following confounding variables: simultaneous exposures to petroleum distillates and/or other solvents; absence of worker smoking histories; and an absence of distinction between laundry and dry cleaning workers which complicates characterization of types and levels of exposure.

Since the time of the Board's review, results have become available from the most complete epidemiology study of the industry conducted to date (Brown and Kaplan, 1987). In this retrospective cohort mortality study of the dry cleaning industry sponsored by the National Institute for Occupational Safety and Health (NIOSH), the authors examined the vital status of 1,690 workers employed for at least one year prior to 1960 at shops where perchloroethylene was the primary solvent. The vital status of cohort members was determined as of December 31, 1982.

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The authors found no increased risk of cancer in a subcohort of 615 dry cleaning workers exposed only to perchloroethylene. In the cohort of workers exposed to other solvents as well, the overall cancer mortality rate was higher than, but not significantly different from, that predicted using U.S. mortality rates. The relative number of cancer deaths among the workers studied was reduced when compared to the higher cancer mortality rates in metropolitan areas investigated. No deaths due to liver cancer were observed. Among the site-specific cancer mortalities, urinary tract cancer (particularly the bladder) was the only one found to have a significant increase. Within the subcohort of workers exposed only to perchloroethylene, the incidence of mortality from urinary tract cancer (and from cancer in general) was lower than that expected based upon overall U.S. mortality rates. In sum, this study shows no increased risk of cancer in dry cleaning workers exposed only to perchloroethylene.

V. Genotoxicity

The draft report mentions (page 3-22) in vitro studies that provide mixed results as to the genotoxicity of synthesized tetrachloroethylene oxide, an epoxide of perchloroethylene. Such results must be interpreted cautiously. Tetrachloroethylene oxide was administered directly to cells in culture at excessive (non-physiological) concentrations that probably overwhelmed the capacity of the cells to detoxify the purported metabolite.

Thus, the reported positive results were generated under artificial physiological conditions with respect to evaluating the likelihood of activity of tetrachloroethylene epoxide.

The extensive genotoxicity data base for perchloroethylene must supercede these in vitro results. Perchloroethylene has consistently shown negative activity in a wide variety of genetic toxicity tests under normal physiologic conditions of metabolite formation. Thus, perchloroethylene is considered to be nongenotoxic by NTP (1986a). Moreover, the Halogenated Organics Subcommittee of the EPA Science Advisory Board specifically addressed the tetrachloroethylene epoxide question (1987):

The Subcommittee disagrees with the statement in the draft Addendum that perchloroethylene is genotoxic by implications because a metabolite of perchloroethylene is genotoxic.

Tetrachloroethylene oxide, the metabolite in question is not a demonstrated metabolite of perchloroethylene but a postulated metabolite, although the assumed pathway is reasonable. The hypothetical conversion of perchloroethylene to tetrachloroethylene oxide does not

appear to account for the carcinogenic properties of perchloroethylene, because perchloroethylene is not mutagenic and because tetrachloroethylene oxide is apparently not carcinogenic.

VI. Weight of the Evidence for Carcinogenicity

It would be scientifically inappropriate not to take all available scientific evidence into consideration in assessing the carcinogenic potential of perchloroethylene. The Executive Summary of the draft report states that EPA is likely to classify perchloroethylene in category B₂, but it is our understanding from recent discussions with EPA staff that the Agency has not reached a final decision as to how to classify the chemical. The conclusion in the 1986 draft EPA Addendum to the Health Assessment Document for Perchloroethylene was not consistent with the Science Advisory Board's review of that document, or with its most recent statements (1988) that the weight of the evidence for perchloroethylene "lies on the continuum between the categories B₂ and C of EPA's risk assessment guidelines." The Board's conclusion should be reflected in the Executive Summary of the draft report and throughout the draft report wherever EPA's classification of perchloroethylene is mentioned.

In its most recent review of perchloroethylene, IARC has concluded that there is not sufficient evidence to warrant a determination that perchloroethylene is probably carcinogenic to humans. Applying the same criteria as IARC results in a determination that perchloroethylene is possibly carcinogenic to humans; it does not justify hazard identification or regulatory action premised on a determination that it is more likely than not that perchloroethylene poses a human cancer risk. Indeed, the change in terminology describing IARC Group 2B was made in part because of the "misuse, or ... exaggeration ... in the use and interpretation of experimental animal results" (Tomatis, 1987).

There are other significant reasons why perchloroethylene should not be identified as posing a cancer hazard to humans. The Guidelines recognize (A-14) that when there is conflicting evidence in several animal bioassays, the positive and negative results should be weighted by the adequacy of the study design, the appropriateness of the species tested, the pharmacokinetics of the species, and the statistical power of the test. The Guidelines further state (A-15) that final conclusions concerning the carcinogenicity of a chemical should be drawn from evaluation of the total body of relevant evidence. Because the nature, extent, and the quality of data concerning carcinogenicity vary widely among different compounds, the evidence of carcinogenicity also varies among them. The final evaluation of a specific chemical must, under the Guidelines, contain an assessment of the

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strength of the evidence as to its carcinogenicity and should also contain a description of the uncertainties underlying the assessment.

The available data on metabolic, mechanistic, and genetic factors, summarized above, has been regarded as highly significant by EPA's Science Advisory Board and others in the scientific community. The Air Resources Board and the Department of Health Services must make a full and fair evaluation of all these data, as part of an overall weight-of-evidence determination as to the potential carcinogenic hazard of perchloroethylene.

VII. Available Pharmacokinetic Information

The risk estimates presented in the draft report range from 5 to 21×10^{-6} for lifetime exposure to 1 ug/m^3 of perchloroethylene. In comparison, EPA has developed unit risk estimates ranging from 2.9 to 9.5×10^{-7} (1986 draft Addendum to the Health Assessment Document for Perchloroethylene). There is, in our view, no good scientific basis for the presentation of 20-fold higher risk estimates, based on default assumptions, than those being considered by EPA.

The draft report should incorporate physiologically-based pharmacokinetic (PB-PK) information in order to develop a more plausible estimate of potential risk. At a public hearing of the

California Air Resources Board concerning the proposed identification of methylene chloride as a toxic air contaminant. Chairwoman Jananne Sharpless stated that such an estimate would "help this Board try to interpret the information on how we go about controlling it" [(July 13, 1989)]. Sharpless also alluded to the upcoming reviews of other chlorinated solvents (i.e., perchloroethylene and others), and expressed her desire on behalf of the Board that a "most plausible" risk estimate be developed. HSIA urges the development of such an estimate by reflecting pharmacokinetic information.

Use of body-surface area correction factors are not appropriate in the case of perchloroethylene. Surface area scaling assumes that humans are more sensitive than rodents, despite the fact that carcinogenic responses in rodents after exposure to perchloroethylene are unlikely to be observed in humans. Body weight provides a better basis for dose adjustment.

In a previous draft assessment of perchloroethylene, the Department of Health Services recognized that "[t]here is extensive information available on the metabolism and uptake [of perchloroethylene] in mammals," and the "[t]he product(s) of this metabolism, rather than PCE molecule per se ... are thought to be responsible ... for the carcinogenicity in laboratory animals." The draft report mentions some of the older pharmacokinetic

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information, but does not address several more recently published works applying a physiologically-based pharmacokinetic (PB-PK) model. While section 5 contains several calculated dose adjustments, the draft report concludes (page 18) that "it appears premature to use the metabolized dose in current estimations of human risk."

The failure to incorporate available published information seems inexplicable, in light of the requirement in the Guidelines (A-16) that "[p]harmacokinetic data on metabolism of dosed substances, effective dose at target site, or species differences between laboratory test animals and humans shall be considered in dose-response assessments when they are available." If the report continues to provide an estimate of potential cancer risk associated with exposure to perchloroethylene in the environment, all available scientific data should be incorporated into the estimate.

Several scientific articles published in the last two years apply PB-PK models to perchloroethylene. These include Ward, et al., Pharmacokinetics of Tetrachloroethylene, Tox. App. Pharmacol. 93: 108-117 (1988); Travis et al., A Physiologically Based Pharmacokinetic Approach for Assessing the Cancer Risk of Tetrachloroethylene, in The Risk Assessment of Environment and Human Health Hazards: A Textbook of Case Studies (Paustenbach, ed), J. Wiley & Sons (1989); and Chen and Blancato, Role of Pharmacokinetic Modeling in Risk Assessment, Perchloroethylene as

an Example, in National Research Council, Pharmacokinetics and Risk Assessment, Drinking Water and Health, Vol. 8, National Academy Press (1987). Copies of these articles are attached. We urge that the final report make use of the available pharmacokinetic information.

VIII. Flaws in Estimates in Draft Report

The range of unit risk estimates presented in the draft report is entirely inconsistent with past human experience. In order to test the predictive value of the risk estimates in the draft report, we have calculated below the risk to individuals occupationally exposed, assuming exposures to have been at or below 200 parts per million (the AGGIH TLV for 1948 to 1981 -- the relevant latency period). Using the upper end of the range presented in the draft report (144 per million at 1 ppb), the risk at 200 ppm is:

$$1 - \exp(-200 \times 1000 \times 144 \times 10^{-6} \times$$

10 cubic meters x 5 days x 49 weeks x 30 years)

----- = 0.98

20 cubic meters x 7 days x 52 weeks x 70 years)

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CORRECTED PAGE

This calculation shows, in other words, a potential risk of 980,000 in 1,000,000. Needless to say, this is a very large and very detectable potential risk. The example of a 30-year exposure at the TLV may be unrealistic, however. Thus, the table presented at the end of our comments shows similar calculations for different fractions of the TLV and for the lower and upper bound of the risk estimates in the draft report.

It can be seen from this table that even the lower bound on the risk estimate range shows extreme (e.g., 7,000 in 1,000,000) potential lifetime risks. Assuming that the exposure scenarios are realistic, either the calculated potential risk is real, does exist, and has gone undetected, or the calculated unit risk estimates are wrong. Past manufacturing plant experience would indicate that particularly the low end of the ppm-year scenarios are very consistent with past practices and may even be underestimates. For example, drycleaning workers, who have a geometric mean exposure of 22 ppm with a skewed distribution, may have an average exposure generally exceeding 25 ppm. Yet the drycleaning industry has been the subject of numerous epidemiology studies, none of which have detected a risk of the magnitude shown in the table. This is true as well for other industries that use perchlorethylene. Thus, we are left with the conclusion that the unit risks estimates in the draft report vastly overestimate the potential risk to humans.

IX. Other Comments

The statement in the Executive Summary that perchloroethylene is used for coffee decaffeination is incorrect. We are not aware that perchloroethylene has ever been used for coffee decaffeination; it is not being used for that purpose currently.

The Executive Summary states that the OSHA PEL for perchloroethylene is 50 ppm for an 8-hour TWA. The OSHA PEL was recently changed and is now 25 ppm for an 8-hour TWA.

The statement on page 1-9 that the EPA range of risks includes the DHS range in the draft report is arithmetically incorrect. The EPA range is 2.9 to 110×10^{-7} , whereas the range in the draft report is 50 to 210×10^{-7} . The interval 50 to 210 is not included in the interval 2.9 to 110.

The statement near the bottom of page 5-2 that metabolized doses lead to higher unit risks than administered doses is mathematically correct, but misleading and irrelevant. Metabolized doses will lead to higher unit risk estimates, but equivalent doses will be smaller, thus risk estimates themselves can be higher or lower, depending upon the metabolism and distribution of the particular compound.

The Q* correcti n on the bottom of pag 5-8 should be clarified to differentiate between exposure period and observation period. The correction is used as a policy decision when the observation period of an experiment is less than lifetime, for example in the first butadiene mouse study. If the dosing is for one year, and the animals are observed for an additional year, as in many of Maltoni's studies, the correction factor is $104/T_e$ raised to the unity power, not the third power.

The comment that inter-individual variability is not accounted for in the PB-PK models is correct, but the same comment could be made for other models, including the LMS model, unless the surface area factor is viewed as an individual tissue sensitivity factor.

TABLE

Exposure Scenario	TABLE	
	UPPER BOUND RISK 144/MILLION per ppb	LOWER BOUND RISK 31/MILLION per ppb
200 ppm 30 years	0.98	0.59
150 ppm 30 years	0.96	0.49
100 ppm 30 years	0.87	0.36
60 ppm 30 years	0.71	0.24
100 ppm 20 years	0.75	0.26
100 ppm 10 years	0.50	0.14
50 ppm 10 years	0.29	0.07
25 ppm 20 years	0.29	0.07

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PERCHLOROETHYLENE
REFERENCES

- Ames, B.N., Morgan, R., Gold, L.S., Ranking Possible Carcinogenic Hazards, Science 236: 271-280 (1987).
- Andersen, M.E., Tissue Dosimetry in Risk Assessment, or What's the Problem Here Anyway? In: Pharmacokinetics in Risk Assessment, Drinking Water and Health, Volume 8, National Academy Press, Washington, D. C. (1987).
- Brown, D.P., and Kaplan, S.D., Retrospective Cohort Mortality Study of Dry Cleaner Workers Using Perchloroethylene, J. Occup. Med. 29: 535-541 (1987).
- Butler, W.H., and Newberne, P.M. (Eds.), Mouse Hepatic Neoplasia, Elsevier, Amsterdam (1975).
- Chen, C.W., and Blancato, J.N., Role of Pharmacokinetic Modeling in Risk Assessment: Perchloroethylene as an Example, In: Pharmacokinetics in Risk Assessment, Drinking Water and Health, Volume 8, National Academy Press, Washington, D. C. (1987).
- Dekant, W., Metzler, M., and Henschler, D., Identification of S-1, 2,2-Trichlorovinyl-N-acetylcysteine as a Urinary Metabolite of Tetrachloroethylene: Bioactivation through Conjugations as a Possible Explanation of its Nephrocarcinogenicity, J. Biochem. Toxicol. 1: 57-72 (1986).
- Elcombe, C.R., Species Differences in Carcinogenicity and Peroxisome Proliferation Due to Trichloroethylene: A Biochemical Human Hazard Assessment, Arch. Toxicol. (Suppl.) 8: 6-17 (1985).
- Fox, T.R., Schumann, A.M., and Watanabe, P.G., Activation of a Cellular Proto-Oncogene in Spontaneous Liver Tumor Tissue of the B6C3F1 Mouse, Arch. Toxicol. 10: 217-227 (1987).

- Fox, T.R., and Watanbe, P.G., Detection of a Cellular Oncogene in Spontaneous Liver Tumors in B6C3F1 Mice, Science 228: 596-597 (1985).
- Goldsworthy, T.L., Lyght, O., Burnett, V.L., and Popp, J.A., Potential Role of Alpha-2u-Globulin, Protein Droplet Accumulation, and Cell Replication in the Renal Carcinogenicity of Rats Exposed to Trichloroethylene, Perchloroethylene, and Pentachloroethane, Toxicol. Appl. Pharmacol. 96: 367-379 (1988).
- Goldsworthy, T.L., and Popp, J.A., Chlorinated Hydrocarbon-Induced Peroxisomal Enzyme Activity in Relation to Species and Organ Carcinogenicity, Toxicol. Appl. Pharmacol. 88: 225-233 (1987).
- Goldsworthy, T.L., Smith-Oliver, P., Loury, D.J., Popp, J.A., and Butterworth, B., Assessment of Chlorinated Hydrocarbon-Induced Genotoxicity and Cell Replication in Rat Kidney Cells, Env. Mol. Muta. 11 (Suppl. 11): 39 (1988).
- Grasso, P., and Crampton, R.F., The Value of the Mouse in Carcinogenicity Testing, Food Cosmet. Toxicol. 10: 418-426 (1972).
- Hazard Evaluation Section, Proposed Maximum Contaminant Level for Tetrachloroethylene in Drinking Water (R-75-87), at 17-23.
- Henschler, D., Elsasser, H., Romer, W., and Eden, E., Carcinogenicity Study of Trichloroethylene, With and Without Epoxide Stabilizers, in Mice, J. Cancer Res. Clin. Oncol. 107: 149-156 (1984).
- Herren-Freund, S.L., Pereira, M.A., Olsen, G., and DeAngelo, A.B., The Carcinogenicity of Trichloroethylene and Its Metabolites, Trichloroacetic Acid and Dichloroacetic Acid, in Mouse Liver, Proc. Amer. Assoc. Cancer Res. 27: 91 (1986).
- International Agency for Research on Cancer (IARC), Monographs, Evaluation of the Carcinogenic Risk to Humans, Supplement 7, Lyon (1987).
- International Expert Advisory Committee to the Nutrition Foundation, The Relevance of Mouse Liver Hepatoma to Human Carcinogenic Risk (1983).
- Moloney, W.C., Boschett, A.E., and King, V.P., Spontaneous Leukemia in Fischer Rats, Cancer Res. 30: 41-43 (1970).
- Moolgavkar, S.H., and Knudson, A.G., Mutation and Cancer: A Model for Human Carcinogenesis, J. Natl. Cancer Inst. 66: 1037-1052 (1981).

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National Cancer Institute (NCI), Bioassay of Tetrachloroethylene for Possible Carcinogenesis, U.S. Department of Health, Education, and Welfare, Bethesda, MD, NIH Publication No. 77-813 (1977).

National Research Council (NRC), Safe Drinking Water Committee, Drinking Water and Health, Vol. 3, National Academy Press, (1980).

National Toxicology Program (NTP), Technical Report on the Toxicology and Carcinogenicity Studies of Tetrachloroethylene in F344/N Rats and B6C3F1 Mice (Inhalation Studies), NTP TR 311, NIH Publication No. 85-2567 (1986a).

National Toxicology Program (NTP), Technical Report on the Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride), NTP TR 306 (1986b).

Odum, J., Green, T., Foster, J.R., and Hext, P.M., The Role of Trichloroacetic Acid and Peroxisome Proliferation in the Differences in Carcinogenicity of Perchloroethylene in the Mouse and Rat, *Toxicol. Appl. Pharmacol.* 92: 103-112 (1988).

Rampy, L.W., Quast, J.F., Leong, B.K.J., and Gehring, P.J., Results of Long-Term Inhalation Studies on Rats of 1,1,1-Trichloroethane and Perchloroethylene Formulation, In: Proceedings of the First International Congress on Toxicology, Plaa, G.L., Duncan, W.A.M. (Eds.), Academic Press, New York (1978).

Schach von Wittenau, M., and Estes, P.C., The Redundancy of the Mouse Carcinogenicity Bioassay, *Fund. Appl. Toxicol.* 3: 631-639 (1983).

Science Advisory Board (SAB), U.S. Environmental Protection Agency, Letter and Report to William D. Ruckelshaus, EPA Administrator (January 4, 1985).

Science Advisory Board (SAB), U.S. Environmental Protection Agency, Letter and Report to Lee M. Thomas, EPA Administrator (January 27, 1987).

Science Advisory Board (SAB), U.S. Environmental Protection Agency, Letter and Report to Lee M. Thomas, EPA Administrator, SAB-EHC-88-011 (March 9, 1988).

Stott, W., Chemically Induced Proliferation of Peroxisomes: Implications for Risk Assessment, *Reg. Toxicol. Pharmacol.* 8: 125-159 (1988).

Tomatis, L., Remarks at the Annual Winter Toxicology Forum (February 1987).

Tomatis, L., Partensky, C., and Montesano, R., The Predictive Value of Mouse Liver Tumor Induction in Carcinogenicity Testing -- A Literature Survey, Int. J. Cancer 12: 1-20 (1973).

Travis, C.C., White, R.K., and Arms, A.D., A Physiologically Based Pharmacokinetic Approach for Assessing the Cancer Risk of Tetrachloroethylene, In: The Risk Assessment of Environmental and Human Health Hazards: A Textbook of Case Studies, Paustenbach, D.J. (Ed.), J. Wiley & Sons, New York (1989).

Ward, R.C., Travis, C.C., Hetrick, D.M., Andersen, M.E., and Gargas, M.L., Pharmacokinetics of Tetrachloroethylene, Toxicol. Appl. Pharmacol. 93: 108-117 (1988).

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

January 27, 1987

Honorable Lee M. Thomas
Administrator
U. S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460

SAB-EHC-87-018

Dear Mr. Thomas:

The Science Advisory Board's Environmental Health Committee has completed its review of a draft Addendum to the Health Assessment Document for Perchloroethylene. The Committee previously reviewed the draft Health Assessment Document on May 9-10, 1984. An Addendum is desirable because of newly available data, primarily an inhalation bioassay of rodents by the National Toxicology Program. The Committee has conducted its review primarily through the Halogenated Organics Subcommittee, whose report is attached.

The Subcommittee believes it is reasonable to describe the weight of the epidemiological evidence in humans as conforming to the EPA guideline for carcinogen risk assessment definition of "inadequate." The Subcommittee concludes that the animal evidence of carcinogenicity is "limited" because of positive results in only one strain of mouse of a type of tumor that is common and difficult to interpret. Thus, the Subcommittee concludes that perchloroethylene belongs in the overall weight-of-the-evidence category C (possible human carcinogen).

Given the current evidence, the Subcommittee hypothesizes that, operationally, perchloroethylene may be an indirect acting carcinogen or carcinogenic promoter of low potency. By promoter, the Subcommittee means that perchloroethylene alone does not induce tumors. Instead, perchloroethylene appears to act in concert with other substances, endogenous processes, viruses, oncogenes, or radiation, which can initiate cancer in the absence of promoters.

We appreciate the opportunity to comment on this important public health issue and request that EPA formally respond to our report.

Sincerely,

Norton Nelson
Norton Nelson

Chair, Executive Committee

Richard A. Griesemer

Richard A. Griesemer

Chair, Environmental Health Committee

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

October 26, 1986

Dr. Richard A. Griesemer
Chair, Environmental Health Committee-
Science Advisory Board
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Dear Dr. Griesemer:

The Halogenated Organics Subcommittee of the Environmental Health Committee has completed its review of a draft Addendum to the Health Assessment Document for Tetrachloroethylene (Perchloroethylene: Updated Carcinogenicity Assessment: EPA-600/8-82/005FA; March, 1986). The Environmental Health Committee previously reviewed the draft Health Assessment Document for Perchloroethylene on May 9-10, 1984, and transmitted a report on this draft to the Agency on January 4, 1985.

The draft Addendum is based on a National Toxicology Program inhalation bioassay of perchloroethylene in rodents. The Subcommittee finds that the bioassay is of reasonably good quality, and that useful results for risk assessment can be obtained from it. The Subcommittee disagrees with the Agency's interpretation of the data that increases in either renal tubular cell neoplasia or mononuclear cell leukemias in F334 rats were associated with perchloroethylene exposure. The Subcommittee agrees with the conclusion in the document that perchloroethylene inhalation is associated with a significant increase in the frequency of liver carcinoma in B6C3F1 mice. This result provides experimental verification of an assumed extrapolation between routes of administration from a gavage study, as described in the Health Assessment Document.

The Subcommittee believes it is reasonable to describe the weight of the epidemiological evidence in humans as conforming to the EPA guideline for carcinogen risk assessment definition of "inadequate." The Subcommittee concludes that the animal evidence of carcinogenicity is "limited" because of positive results in only one strain of mouse of a type of tumor that is common and difficult to interpret. Thus, the Subcommittee concludes that perchloroethylene belongs in the overall weight-of-the-evidence category C (possible human carcinogen).

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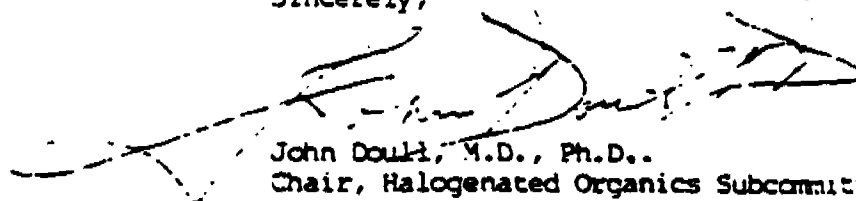
In the opinion of some members of the Subcommittee, a quantitative assessment of perchloroethylene is desirable, and the mouse data are adequate for this purpose. Treatment of this assessment as a "what-if" calculation, as presented in the original Health Assessment Document, is desirable. Such a quantitative assessment probably will show that an increase in cancer would not be detected in the groups most exposed to perchloroethylene at current exposure levels. This inference deserves mention in the executive summary. The analysis of pharmacokinetics in the draft Addendum is commendable and will support further estimates of the possibility of cancer in exposed populations.

The Subcommittee requests that the Environmental Health Committee address the question of whether one-tailed or two-tailed statistical tests of significance are appropriate for the routine analysis of bioassay data. Agency staff reported at the meeting that one-tailed tests are routinely used. This use assumes that chemical substances can only increase the frequency of cancer, but this assumption seems contrary to empirical observations. Resolution of this issue will influence the conclusions regarding perchloroethylene. In addition, the Subcommittee requests that the Environmental Health Committee arrange for a detailed review of the physiological-pharmacokinetic model used in the analysis of perchloroethylene. The Subcommittee reviewed some results of the model but not the model per se. EPA is likely to use the model in the future to assess the risks of other substances.

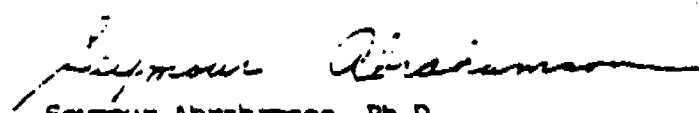
In support of the review of perchloroethylene, the Subcommittee requests that the Agency provide the members with analyses of (1) human carcinogens and their effects in the rat and mouse, (2) human hepatotoxins and their effects in the rat and mouse, and (3) human renal toxins and their effects in the rat and mouse.

The Subcommittee believes that the final Addendum will enhance the value of the Health Assessment Document and that, contingent on the correction of the issues discussed in the attached report, the document will be scientifically adequate to meet its stated purposes. We appreciate the opportunity to comment on this public health issue and request a formal response to our advice.

Sincerely,



John Doult, M.D., Ph.D.,
Chair, Halogenated Organics Subcommittee



Seymour Abrahamson, Ph.D.
Vice-Chair, Halogenated Organics Subcommittee

REPORT OF THE HALOGENATED ORGANICS SUBCOMMITTEE OF THE
ENVIRONMENTAL HEALTH COMMITTEE ON A DRAFT ADDENDUM TO THE
HEALTH ASSESSMENT DOCUMENT FOR TETRACHLOROETHYLENE (PERCHLOROETHYLENE)

Introduction

The Halogenated Organics Subcommittee of the Environmental Health Committee of EPA's Science Advisory Board met on May 15, 1986 in Madison, Wisconsin, to review a draft Addendum to the Health Assessment Document for Tetrachloroethylene (Perchloroethylene; Updated Carcinogenicity Assessment; EPA-600/8-82/005FA; March, 1986). The Environmental Health Committee previously reviewed the draft Health Assessment Document for perchloroethylene on May 9-10, 1984. A report on this draft was sent to the Agency on January 4, 1985. The draft Addendum primarily analyzes the results of a 1985 inhalational bioassay by the National Toxicology Program performed at Battelle Pacific Northwest Laboratories. The Subcommittee thanks the National Toxicology Program for sending a representative, Dr. Jonn Minnear, to contribute to the discussion at the May 15th meeting.

The Subcommittee concludes that the Addendum improves the scientific foundation of the existing Health Assessment Document and further improves the Agency's ability to perform a risk assessment for this compound. The Agency intends for the Health Assessment Document to serve as a multimedia source document. The Subcommittee believes that, contingent on the correction of the issues discussed below, the draft Addendum will also be scientifically adequate for this purpose.

Quality Assurance of the Bioassay

The Subcommittee reviewed the available quality assurance information of the bioassay. It concludes that these audits were properly carried out and generally are consistent with each other. The quality assurance process identified discrepancies during the collection of in-life toxicology data, verification of analytical chemistry results, cage injuries, brief overexposure of the high dose group of rats, autolysis of some specimens, and the failure to section all lesions. Some animal escapes and confusion over animal identification were reported. However, there is little possibility that animals moved from control to test cages, or between test groups, because the animal housing in the Battelle inhalation chamber is well controlled, and the chambers are in rooms within a barrier facility. The Subcommittee also does not believe that resorting a few animals would likely create a positive result as an artifact with a rare tumor. The random movement of a few animals between cages is more likely to obscure the detection of a statistically positive finding of an infrequent lesion.

These problems are fairly common in bioassay work and do not impede the interpretation of clear, distinctive findings. The Subcommittee believes that the bioassay provided data that are adequate for risk assessment, unless EPA attempts to interpret small differences between groups of animals.

The Subcommittee recommends that the Agency summarize and assess the implications of the quality assurance information in the final Addendum.

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Interpretation of the Carcinogenicity Results

The draft Addendum describes two lesions of potential interest that occurred in Fisher 334/N rats--renal tubular neoplasia and mononuclear cell leukemias. The Subcommittee concludes that the National Toxicology Program bioassay does not provide a scientific basis to associate either lesion with inhalational exposure to perchloroethylene. Both findings would result from small differences between control and treated groups, and they conflict with other bioassays of perchloroethylene in the rat and which are problematic in relation to the quality assurance conclusions. Both findings have multiple problems, any one of which overwhelms the interpretation. These problems include:

a) Perchloroethylene neither appeared to induce an increase in rare renal tubular neoplasia in male rats, nor was the trend in these tumors among the male rats dose-related. No renal tubular neoplasia were observed in female rats. The reported numbers of adenomas in male rats were 1/49 (control), 3/49 (200 ppm) and 2/50 (400 ppm). Renal tubular carcinomas occurred in 0/49 (control), 0/49 (200 ppm) and 2/50 (400 ppm) of the male rats. When results of both tumors are reported, the numbers of animals affected were 1/49 (control), 3/49 (200 ppm) and 4/50 (400 ppm). To attribute statistical significance to the findings in male rats, the analysis aggregated adenomas and carcinomas. However, the analysis of numbers of animals with adenomas or carcinomas as a group is not an obvious biological procedure.

The pathology of these tumors is not well-understood, and little background information is available in the literature. The diagnosis of renal tubular neoplasia in the rat is not a clearly understood procedure among experts. Whether or not conversion from adenoma to carcinoma occurs is not known, and the draft Addendum does not review this subject. In addition, the statistical analyses supporting conclusions in the text are in error. The Fisher exact test has been miscalculated, and trend has not been analyzed.

The Subcommittee recommends that the Agency develop better descriptions of (1) the pathology of the renal tubular neoplasia in rats (including speculations about progression or conversion), and (2) the rationale for aggregating the numbers of animals with adenomas or carcinomas. At a minimum, it should assess each data set independently before evaluating the aggregated data, and the results of statistical tests for any trends. For the benefit of potential non-expert readers, the final Addendum needs to clarify that the enumeration of rats with carcinoma or adenoma is subject to debate.

To analyze the renal tubular neoplasia results, the Agency has to address several competing hypotheses, such as an unusual occurrence within: the specific group of F334 rats used in the bioassay, aberrant housing conditions, historical underdetection of renal tubular neoplasia, induction of tumors by perchloroethylene, and so forth. Eight (8) out of 148 (5.4%) male rats had findings of adenoma or carcinoma. Either this frequency or the frequency for all untreated rats, male and female (8/296; 2.7%) can be compared to the reported historical frequency of 4/1,720 or 0.23%. (The note in the draft Addendum does not clearly state the basis of the historical observations.) The Addendum also needs to address the credibility of this number in the light of the probably variable search for lesions in the absence of cross observations that suggest a neoplastic response. Historically, renal tubular neoplasia in control rats tend to be under-reported.

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Questions can be asked and then answered, about possible biological or statistical reasons for the differences in control and overall incidence. The Subcommittee suggests that the staff calculate the prior likelihood of the frequency of renal tubular neoplasia under different hypotheses about the average rate of occurrence, using the Poisson distribution. The staff can compare these results to each other and to the biological interpretations of each hypothesis.

b) Perchloroethylene did not appear to induce a marginal increase in mononuclear cell leukemia in rats. At the present time, the scientific community has a poor understanding of the pathology of mononuclear cell leukemia. The high frequency in all groups, including controls is not usual for F334 rats. The results suggest faulty pathological diagnoses or some unusual circumstances in the rat colony at the time. The extent of characterizing mononuclear cell leukemias was histopathological examination. Other means of characterization, which are necessary to distinguish neoplasia from leukocytic hyperplasias that may develop in older rats, were not used.

The results of the statistical analysis are not convincing. The Addendum presents the mononuclear cell leukemia data in terms of a progressive three stage classification which appears to be preliminary and ad hoc. The staging of diagnoses does not represent a consensus effort of the community of experienced pathologists. However, the draft Addendum states that the strength of the evidence for carcinogenicity in the F334 rat rests on the resolution of issues regarding the uncertainty in the assignment of frequency within the stages of mononuclear cell leukemia. No human analogue is known for mononuclear cell leukemia of the rat. This absence is not important for EPA's policies on carcinogenicity, although a lack of correspondence does concern some biologists. However, the absence of a human analogue is important, when staging is considered, since staging refers to the usually more extensive information on leukemic progression in humans.

If a two-tailed test is used, the most striking observation in the results occurred at 200 ppm, in which 12 of 50 female rats in the control group were reportedly diagnosed as having mononuclear cell leukemia in one of the three stages versus 30 of 50 of the perchloroethylene treated female rats. This comparison leads to a confidence limit of about $p = 0.03$ by the Fisher exact test (two-tailed). Regardless of the statistic, 12 versus 30 is not a striking observation and, given the generally high frequency of mononuclear cell leukemia diagnoses in all groups, it is worth inquiring what the chance is of finding such a result if two of six groups are drawn at random, each group being subject to the same high, random frequency of diagnoses (reported as 179 of 300 or about 60%).

In oral statements at the meeting, Agency staff reported that a statistically significant difference between untreated and perchloroethylene treated rats could be observed for mononuclear cell leukemia, if the time-to-tumor was analyzed. This may be the case, but these oral statements contradict the written statements in the draft Addendum regarding time-to-tumor. Some Subcommittee members have attempted to evaluate whether or not diagnosable mononuclear cell leukemia occurred earlier in perchloroethylene exposed rats than in unexposed.

The presentation of data in the draft Addendum is such that this comparison cannot be made because neither the actual data, nor the dependent probabilities, are presented. (See "statistical analyses," below.) However, the draft Addendum states anecdotally at several points that this comparison is not worth making because of analyses contained in the National Toxicology Program report.

The draft Addendum does not review the analyses, and does not present the supporting data. However, if the oral comments are correct, then either the National Toxicology Program analysis, or the interpretation of this analysis in the draft Addendum, is in error.

The Subcommittee suggests that the Agency will experience difficulty in gaining scientific support for the conclusion that perchloroethylene exposure is associated with increased frequency of mononuclear cell leukemia in rats, based on the National Toxicology Program bioassay data. Any effort to do so should begin with an explanation of why the frequency of this tumor did not increase after perchloroethylene exposure of rats in the bioassays performed by the National Cancer Institute and by Rampy and co-workers.

The Subcommittee agrees with the statement in the draft Addendum that first generation hybrid mice of C57B16 and C3H parental origin (B6C3F1) exhibit statistically significant increases in carcinoma of the liver associated with exposure to perchloroethylene by inhalation. These results confirm the findings of a National Cancer Institute study with the same strain of mouse and administration of perchloroethylene by gavage. The Environmental Health Committee and its Subcommittees have consistently urged the Agency to calculate the potency of a carcinogen for all routes of administration when data are available for only one route (using the best general information about uptake, absorption, metabolism, distribution, elimination and mechanism). Once data has existed for one route, it has advised EPA to use the empirical evidence as the basis for decision-making and to forgo the hypothetical calculation. Perchloroethylene provides an example of experimental validation, both qualitatively and quantitatively, of the hypothetical extrapolation. However, this validation does not change the interpretation on which a decision might be based. No new, dispositive information has been gained.

Although the possibility exists that carcinomas arise de nova, the available evidence strongly supports the idea that the adenomas and carcinomas represent a single disease process to which scientists have applied an arbitrary division into two diagnostic terms. Since we usually don't know the rate at which the various lesions progress after exposure to a given test chemical, and because histologic evidence alone is not entirely a satisfying indicator of biological behavior, the Subcommittee recommends analyzing the lesions both separately and combined. It should be remembered that many mice with hepatic carcinomas also have adenomas that have not been included in the summary tallies.

Other Data from the National Toxicology Program Bioassay

The Subcommittee requests that Agency staff fully assess all of the information available from the National Toxicology Program study. The draft Addendum notes the occurrence of squamous cell metaplasia of the nasal cavity in male rats but does not provide statistical analysis of significance or trend with dose. The draft Addendum refers to a finding of renal tubular cell hyperplasia in rats, but no data are provided. Renal tubular karyomegaly is noted in rats and mice of both sexes, but no data are provided.

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The Subcommittee also recommends that Agency staff thoroughly assess and interpret the significance of mortality outcomes for rodents chronically exposed to perchloroethylene in the National Toxicology Program bioassay. These data can be important in setting standards for drinking water. One interesting possibility is that the kidney also is a target organ. The draft Addendum notes excess mortality in mice at 100 ppm and 200 ppm but suggests that this result is caused by hepatic cancer. An appropriate statistical analysis of mortality will correct for this effect by correcting for deaths from hepatic cancer (a time-to-not-tumor calculation).

Statistical Analysis of the Bioassay Results

The display of data and statistical analysis of these data in the draft Addendum needs revision. The Subcommittee found some critical instances of misquotation and error.

While the statistical analyses reported in the Addendum can be reproduced by the Subcommittee, this can only be done if a one-tailed Fisher exact test is used. The use of a one-tailed test is appropriate, if perchloroethylene only can increase the frequency of cancer. This assumption is dubious when the background in the control group is high, and it is contrary to the general knowledge about the effects of chemicals on tumor frequency in rodents.* Instead, a two-tailed test seems appropriate. The Agency should state whether an analysis is one-tailed or two-tailed in the text.

Metabolism and Pharmacokinetics

The Subcommittee believes that the draft Addendum and the final Health Assessment Document provide a thoughtful response to the comments regarding pharmacokinetics made during the previous review. The data in the draft Addendum are adequate for evaluating potential metabolic mechanisms which pertain to possible carcinogenic effects perchloroethylene. Further, the Subcommittee commends the Agency for the discussion of the different mechanistic implications of perchloroethylene metabolites in the induction of cancer.

At present, the Subcommittee has only reviewed some results of the model used by Agency staff to analyze data for perchloroethylene. Because of the potential importance of such models for EPA risk assessments, the Subcommittee recommends that the Environmental Health Committee undertake a review of the general approach. However, the Subcommittee has developed a consensus regarding one issue that was subject to contention during the public meeting. EPA has not double counted the factor for interspecies extrapolation of metabolized dose. Because staff have modeled the absolute amount per unit volume (tissue specific concentration), some extrapolation between species is required. However, the Agency loses some of the power of the physiological-pharmacokinetic models when this approach is taken.

* See, for example, J.K. HASEMAN, "Patterns of Tumor Incidence in Two-year Cancer Bioassay Feeding Studies in Fisher 334 Rats," Fundamental and Applied Toxicology 3 (1983), pp. 1-9.

Because of the implication that tetrachloroethylene oxide is a carcinogenic intermediate, discussion of the reactivity of various haloethylene oxides should be included. Agency staff should search for studies which correlate the chemical reactivity, hepatotoxicity and carcinogenicity of haloethylene oxides, such as those by Henschler or Van Duuren.

Most studies have attributed the metabolism of perchloroethylene to a proposed reactive metabolite, tetrachloroethylene oxide, which is converted by rearrangement to trichloroacetyl chloride. The latter will acylate rather than alkylate macromolecules. The acylation reaction could be followed by spontaneous hydrolysis and regeneration of the free macromolecules. Thus, no genetic effect may be observed. Indeed, Van Duuren and coworkers have concluded from their studies of the carcinogenicity of various halo-substituted ethylene oxides that tetrachloroethylene oxide is not carcinogenic when administered to rats by any of several routes.

The discussion in the addendum suggests that tetrachloroethylene oxide is the only reactive, carcinogenic metabolite formed following perchloroethylene administration. The Subcommittee recommends that other putative carcinogenic metabolites be described. For example, glutathione conjugation products should also be included. The role of these potential metabolites in eliciting effects, such as renal damage or carcinogenicity, should be discussed. Henschler has suggested glutathione conjugates of various haloethylene compounds as the proximal initiators of renal toxicity, particularly after hydrolysis in the kidney renal tubule.

Several authors have described the covalent binding of radioactive perchloroethylene to tissues after metabolic activation. This binding may be partially due to the formation of acyl derivatives after the formation of trichloroacetyl chloride as an intermediate, as suggested by studies in which trichloroacetic acid was found after acidic hydrolysis of labelled macromolecules. The significance of the acylation reaction in genotoxicity is not clear. However, no covalent binding to deoxyribonucleic acid has been demonstrated, which is indicative of a protective or hydrolytic mechanism, perhaps accelerating the decomposition of tetrachloroethylene oxide to trichloroacetyl chloride before the oxide can gain access to deoxyribonucleic acid. Trichloroacetyl chloride can react with macromolecules to form various trichloroacetic acid esters which may undergo rapid enzymatic hydrolysis to yield trichloroacetic acid and regenerate the macromolecules. This hypothesis merits investigation.

Genotoxicity

The Subcommittee disagrees with the statement in the draft Addendum that perchloroethylene is genotoxic by implication because a metabolite of perchloroethylene is genotoxic. Tetrachloroethylene oxide, the metabolite in question, is not a demonstrated metabolite of perchloroethylene but a postulated metabolite, although the assumed pathway is reasonable. The hypothetical conversion of perchloroethylene to tetrachloroethylene oxide does not appear to account for the carcinogenic properties of perchloroethylene, because perchloroethylene is not mutagenic and because tetrachloroethylene oxide is apparently not carcinogenic. Perchloroethylene has been tested in many mutagenicity bioassays, a few of which show positive activity, but on balance the weight-of-the-evidence is borderline and not conclusive.

Mechanism

Given the current evidence, the Subcommittee hypothesizes that, operationally, perchloroethylene may be an indirect acting carcinogen or carcinogenic promoter of low potency. By promoter, the Subcommittee means that perchloroethylene alone does not induce tumors. Instead, perchloroethylene appears to act in concert with other substances, endogenous processes, viruses, oncogenes, or radiation, which can initiate cancer in the absence of promoters. Initiators are usually thought to be genotoxic substances, binding to deoxyribonucleic acid in order to cause initiating events. When perchloroethylene is present, however, tumors are observed when they would not otherwise be, even when the initiator is not known. Although definitive evidence is lacking, perchloroethylene appears to act at a later stage in the carcinogenic process.

The evidence which leads to the Subcommittee's hypothesis that perchloroethylene may act as an indirect acting carcinogen or a promoter is that perchloroethylene: (1) probably is not mutagenic; (2) does not bind to deoxyribonucleic acid; (3) increases the frequency of liver carcinomas in B6C3F1 mice when these tumors are commonly seen in the same strain not exposed to perchloroethylene; (4) induces liver carcinoma in a species and strain specific manner; (5) induces peroxisomes in the livers of B6C3F1 mice, which provides an alternative mechanism; and (6) acts consistently in comparative studies of halo-substituted ethylenes which indicate that asymmetrically substituted compounds generally are carcinogenic, whereas symmetrically substituted generally are not.

Epidemiology

The Environmental Health Committee has previously reviewed the epidemiological evidence as it was discussed in the Health Assessment Document. The Subcommittee finds no reason to alter the Committee's previous findings at this time. The National Cancer Institute may publish a new epidemiology study of perchloroethylene. The Subcommittee recommends that the Agency evaluate these results in the Addendum, if they are available in a timely and satisfactory form.

Weight-of-the-evidence Category

Based on the National Toxicology Program bioassay results and the Agency's guidelines for carcinogen risk assessment, the Subcommittee concludes that "limited" evidence exists for the carcinogenicity of perchloroethylene in animals because the evidence arises only from a single strain of mouse and because the kind of tumor associated with perchloroethylene exposure in this mouse strain makes it difficult to create an inference regarding human carcinogenicity. The epidemiological evidence is described in the Health Assessment Document as "inadequate." Working from EPA's proposed guidelines, the Subcommittee concludes that the overall weight-of-the-evidence category is C ("possible human carcinogen"). The Subcommittee has carefully considered and rejected the position of some staff that positive evidence of liver carcinoma in the B6C3F1 mouse associated with exposure to perchloroethylene by two different routes of administration should change the weight-of-the-evidence category to B ("probable human carcinogen").

* See A. BLAIR, P. TOLBERT, T. THOMAS and D. GRAUMAN, (Abstract) Mortality among Dry Cleaners. Fourth International Symposium on Epidemiology in Occupational Health (September 10-12, 1985).

Retrospective Cohort Mortality Study of Dry Cleaner Workers Using Perchloroethylene

David P. Brown, MPH, and Samuel D. Kaplan, MD

To evaluate the carcinogenic potential from occupational exposure to perchloroethylene (PCE), a retrospective cohort mortality study of workers employed in the dry cleaning industry was conducted among 1,690 workers from four labor unions. The majority of the cohort had potential exposure to petroleum solvents as well as to PCE while working in the dry cleaning industry. Mortality from primary cancer of the liver was of particular interest, due to the findings of excess liver cancer in mice exposed to PCE. Other sites of cancer were also of interest.

A total of 493 deaths were observed, whereas 573.5 were expected based on US mortality rates. Mortality from all cancers combined was greater than expected (145 observed v 122.9 expected). No deaths due to liver cancer were observed. Urinary tract cancer was the only specific site where there was a statistically significant excess in observed deaths (12 observed v 4.7 expected). There was some consistency in these findings across the four individual unions and across race/sex groups. A subcohort of workers who were employed only in dry cleaning shops that used PCE as their primary solvent was identified from the union records. There was only one death from urinary tract cancer, whereas 1.3 deaths were expected in this subcohort.

Perchloroethylene (PCE), also known as tetrachloroethylene, is a solvent used commonly for cleaning fabric (dry cleaning) and for degreasing metals. It has been estimated that at least 1.6 million workers in the

United States are potentially exposed to PCE.¹ The possibility that PCE could pose a significant occupational health risk to these workers was raised after a National Cancer Institute (NCI) bioassay indicated that PCE induced liver tumors in exposed mice.² In addition, toxic tubular nephropathy was observed in the treated mice.

In an unpublished study conducted by The Dow Chemical Company in 1977, there was no evidence of a tumorigenic response in rats exposed by inhalation to PCE. There was increased mortality among the rats in the high-dose group.

The National Toxicology Program (NTP)³ recently completed a study where F344/N rats and B6C3F₁ mice were exposed to PCE by inhalation. PCE produced renal tubular cell karyomegaly, which is an abnormal enlargement of the cell nucleus, and renal tubular cell hyperplasia in rats. It also increased the incidence of renal tubular cell adenomas or adenocarcinomas in male rats. Both low and high doses of PCE were associated with an increased incidence of mononuclear cell leukemia in male rats and in female rats the low dose increased the incidence of leukemia. In mice there was a dose-related increase in the incidence of hepatocellular neoplasms. PCE also produced renal tubular cell karyomegaly in mice.

Due to the potential carcinogenic effect of PCE as demonstrated by the animal studies and due to the widespread use of PCE in the workplace, an epidemiologic retrospective cohort mortality study was conducted to examine the effects on exposed workers, particularly the risk of mortality from cancer. After evaluating the numerous occupational groups potentially exposed to PCE, the dry cleaning industry was chosen for the epidemiologic study, because PCE had been used as a cleaning solvent in this industry for at least 30 years.

PCE was introduced into the dry cleaning industry in the late 1930s but did not replace other synthetic sol-

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vents such as carbon tetrachloride until shortly after world War II. During this same period petroleum derivatives (primarily various types of Stoddard solvents) were the predominant solvents used in dry cleaning. A gradual shift from petroleum derivatives to PCE began in the late 1940s. This shift in solvents increased in the 1950s and early 1960s. However, in the period before 1960, petroleum derivatives were still the dominant solvents. By 1977, the industry estimated that approximately 74% of commercial dry cleaning shops used PCE, about 24% used petroleum solvents, and the remainder used fluorocarbons (personal communication from William Fisher, International Fabricare, 1985).

An exposure evaluation survey, which included a random sample of the facilities (still in business) in the epidemiologic study, was conducted from 1977 to 1979.⁶ There were 44 commercial dry cleaning shops included in the survey. Time-weighted average (TWA) and peak exposures to PCE were determined by collecting personal air samples. Other solvents used for spot removal were also sampled. The operator or dry cleaner had exposures significantly higher than the other workers (geometric mean TWA of 22 ppm v approximately 3.0 ppm, respectively). Based on historical exposure data some of which date back to 1956, the levels of exposure to PCE in commercial dry cleaning shops have remained fairly constant since its introduction into the industry. The survey also revealed that there was consistency in the level of exposures by geographic location. The only substance detected in the air samples during the survey was PCE. Therefore, even though other solvents were used for spot removal, their airborne concentrations were nondetectable.

Methods

The study cohort was defined to include workers exposed to PCE for a minimum of 1 year prior to 1960, and with no known previous occupational exposure (in the dry cleaning industry) to carbon tetrachloride or trichloroethylene.

Records maintained by four local unions were used to identify dry cleaner workers who met the definition of the study cohort. The majority, if not all, of the dry cleaning shops were commercial as opposed to industrial cleaners. Workers were chosen only when there was documentation that they were employed for at least 1 year prior to 1960 at a shop where PCE was the primary solvent. Some and/or a complete solvent history was available for approximately half of the shops employing union members. If no solvent history was available for a particular shop, employment in that shop was not considered in determining the eligibility of union members. For each eligible worker a history of employment in PCE as well as non-PCE dry cleaner shops was coded. If solvent history was unknown prior to 1960, it was assumed to be non-PCE inasmuch as most shops used petroleum solvents prior to this date.

In an attempt to restrict an analysis to a cohort of workers primarily exposed to PCE with no confounding

exposure to petroleum solvents, a subcohort of workers who were known to be employed only in shops where PCE was the primary solvent was identified.

The vital status of cohort members was determined as of Dec 31, 1982. For those identified as deceased, copies of their death certificates were obtained and the underlying cause of death was coded by a trained nosologist according to the Revision of the International Classification of Diseases (ICD) in effect at the time of death. Those lost to follow-up (unknown vital status) and those who died subsequent to the closing date of the study, i.e. Dec 31, 1982, were considered alive for purposes of analysis.

Person-years at risk (PYAR) were calculated for each worker starting after 1 year of employment in a PCE shop(s) and ending at the date of death or the closing date of the study, whichever occurred first. Using a life-table analysis system,⁷ the PYAR for each worker were combined into 5-year calendar time periods and 5-year age groups. PYAR were additionally distributed by length of employment and by time since first employment in PCE shops (latency). Employment in unknown or petroleum solvent shops was not used in the calculations of length of employment or latency.

The PYAR stratified into age and calendar time periods were multiplied by the corresponding US mortality rates to yield expected numbers of deaths. At the time of this study, the life-table analysis system only maintained US mortality rates through 1978, the end of the eighth revision of the ICD. To calculate expected deaths through 1982 for this study the death rates for the interval 1975 to 1979 were based on US deaths occurring through 1978, and the death rates for the interval 1980 to 1982 were assumed to be identical to the previous time period (1975 to 1979).

The observed and expected cause-specific deaths were compared and differences were tested assuming the Poisson distribution.⁸ The risk is reported as a standardized mortality ratio (SMR), defined as observed/expected deaths $\times$ 100.

Because each of the unions included in the study were located in large metropolitan cities where the mortality rates from cancer are generally higher than those of the total US, state mortality rates corresponding to the location of each union were also used in calculating expected cancer deaths. The state mortality rates more closely estimate the rates of the cities.

Results

The cohort totaled 1,690 workers with 493 deaths and contributed 42,267 PYAR to the analysis. The vital status follow-up through Dec 31, 1982, was successful for 93% (i.e. 7% lost to follow-up) of the cohort. The follow-up for females (92%) was much less complete than for males (97%). This is primarily due to name changes and women dropping out of the work force at an earlier age. Therefore, they are less likely to be listed in the Social Security Administration records.

The subcohort of workers employed only in shops

where PCE was the primary solvent totaled 615: 113 white males, 94 non-white males, 195 white females and 213 nonwhite females. There were a total of 137 deaths identified in this subcohort.

Although all sites of cancer and particularly cancer of the liver were the causes of greatest interest, multiple causes of death were also examined. Table 1 lists the observed and expected deaths for most major diseases and for specific sites of cancer. As seen in most cohort studies of workers, the observed number of deaths for all causes is less than expected (493 observed v 575.5 expected; SMR = 86) with statistically significant deficits in diseases of the circulatory system and diseases of the nervous system (primarily stroke), demonstrating the healthy worker effect. The number of observed deaths for all neoplasms is higher than expected (142 observed v 122.9 expected; SMR = 116). No deaths from liver cancer were observed, whereas 3.5 were expected. Only urinary tract cancer showed a statistically significant excess in observed deaths (12 observed v 4.7 expected; SMR = 255). Within the urinary tract cancer category, both kidney and bladder cancer were elevated with bladder cancer being statistically significant (8 observed v 2.7 expected; SMR = 296).

Other interesting results among the malignant neoplasms include cancer of the cervix uteri which shows an elevated SMR (10 observed v 5.1 expected; SMR = 196) and cancer of the breast which is slightly lower than expected (12 observed v 13.8 expected; SMR = 87). These results probably reflect the socioeconomic status of the cohort which is generally lower income. This type of pattern has been shown in other studies.⁷

In the analysis of cancer mortality based on death rates of the state where each union was located (rather

than the US death rates), there are 140.2 expected deaths from all cancer sites which is closer to the 142 deaths observed in the study population. However, except for pancreatic cancer (11 observed v 10.7 expected), there was very little change in the expected number of deaths from the specific sites of interest. For urinary tract cancer the expected number of deaths based on state rates is 4.9, v 4.6 based on US rates. Therefore, all remaining results in this manuscript are based on expected deaths calculated using US death rates.

Mortality from all major organ systems (other than cancer) was lower than expected, except for a slight excess in diseases of the digestive system (22 observed v 18.8 expected; SMR = 117). Mortality from all accidents is significantly lower than expected (3 observed v 23.9 expected; (SMR = 13). Almost all categories of accidents showed a deficit in mortality, with transportation accidents accounting for the largest portion of the overall category (0 observed v 10.7 expected).

Mortality by race and sex group was examined separately. For all groups, overall mortality was lower than expected, and cancer mortality was higher than expected. In nonwhite males the excess in cancer mortality was statistically significant. In three out of the four race/sex groups mortality from bladder and kidney cancer was elevated, although these were based on small numbers of observed and expected deaths. For bladder cancer a statistically significant excess was observed in nonwhite males (3 observed v 0.6 expected; SMR = 500). Breast cancer was lower in both racial groups and cervix uteri cancer was higher than expected. In all four groups mortality from accidents was significantly lower than expected. In white males there was an un-

TABLE 1
Cause-specific Mortality of Dry Cleaner Workers From All Four Unions Included in Cohort (All Race and Sex Groups)^a

Cause	Observed	Expected	SMR	95% CI
All malignant neoplasms (MN)	142	122.9	116	97-136
MN of buccal cavity and pharynx	3	2.9	103	21-302
MN of digestive organs and peritoneum	38	35.5	107	76-147
MN of intestine except rectum	18	11.8	136	78-220
MN of liver	0	3.5	—	—
MN of pancreas	11	6.4	172	86-310
MN of respiratory system	29	25.4	114	76-164
MN of breast	12	13.8	87	45-152
MN of female genital organs	16	12.7	126	72-204
MN of cervix uteri	10	5.1	196	95-363
MN of male genital organs	8	5.5	145	63-286
MN of urinary organs	12	4.7	255	132-450
MN of kidney	4	2.0	200	55-517
MN of bladder	8	2.7	296	128-586
MN of other and unspecified sites	20	13.1	153	93-236
MN of lymphatic and hematopoietic tissue	4	9.3	43	12-110
Diseases of the nervous system	44	60.5	73	53-98
Diseases of the circulatory system	163	232.0	70	60-82
Diseases of the respiratory system	23	28.7	80	51-120
Diseases of the digestive system	22	18.8	117	73-177
Cirrhosis of the liver	14	12.9	109	60-183
Diseases of the genitourinary system	10	10.2	98	47-180
Calculus of the urinary system	2	0.3	667	73-2186
Accidents	3	23.9	13	3-37
Violence	10	12.4	81	39-149
All deaths	493	575.5	86	78-94

^a Abbreviations used are: SMR, standardized mortality ratio; CI, confidence interval.

usual finding within diseases of the genitourinary system: a statistically significant excess in mortality from calculi of the urinary system was found (2 observed v 0.09 expected).

Mortality was also examined across individual unions which represent totally independent cohorts. Consistent with the findings of the combined cohort, a deficit in overall mortality and an excess in cancer mortality was noted across all four unions. There was also a deficit in accidents in all four unions. In three of the four unions there was an excess in bladder cancer, two of which were statistically significant. Two of the four unions had an excess in kidney cancer. All unions had an increase in cancer of the intestine. The SMRs by race and sex groups and by union for selected causes of death are summarized in Tables 2 and 3.

Analyses for cancer of the intestine and bladder by length of employment in PCE shops (a surrogate of exposure) and by time since first exposure (latency) were conducted to aid in the interpretation of the results. These analyses are given in Tables 4 and 5. There appears to be a positive trend of increasing risk with an increase in latency and exposure for both cancer sites. Separate analyses for kidney cancer are not presented because of the small numbers.

In the subcohort of workers who were employed only in shops where PCE was the primary solvent, there was only one death from urinary tract cancer, whereas 1.3 were expected. Both of the deaths from renal calculi were included in this subcohort, whereas only 0.09 deaths were expected.

An attempt was made to confirm the cause of death for each of the bladder and kidney cancer cases. Medical histories and pathology reports were requested from the hospital where the death occurred. Information was obtained for six of the eight bladder cancers and one of the four kidney cancers. The cause of death from the death certificate was confirmed in each case where information was available from the hospital.

Discussion

The diseases of concern in this study were all sites of cancer, especially cancer of the liver. Liver cancer was of particular interest because of the NCI bioassay and the recent NTP study which demonstrated that mice

exposed to PCE developed liver tumors. The NTP study also demonstrated the PCE may be associated with renal carcinogenicity and leukemia in rats. Because it is not clear how an animal carcinogen might express itself as a human carcinogen, all other sites of cancer were of interest as well.

Mortality from all causes was found to be less than expected, which is probably due to the "healthy worker effect." Mortality from all cancers was higher than expected, almost reaching statistical significance—95% confidence interval for the SMR was 97 to 136. The excess in all cancers was a consistent finding across all race and sex groups and all unions; however, this excess was reduced when state mortality rates were used for calculating expected deaths.

Urinary tract cancer was the only specific site found to have a statistically significant excess in observed deaths. This excess was primarily due to bladder cancer; however, kidney cancer was also found in excess. There was some consistency in this finding in that bladder cancer was elevated in three out of the four race/sex groups, and in three out of the four unions, although

TABLE 3
Standardized Mortality Rates for Selected Causes by Union

Cause of Death	Union 1	Union 2	Union 3	Union 4
All causes	71	83	84	96
All malignant neoplasms	132	124	103	106
MN of intestine	167	115	136	156
MN of pancreas	167	214	83	167
MN of kidney	—†	222	—	333
MN of bladder	1,000*	416*	200	—
Accidents	—	10*	—	—

* $P < .05$.

† — = no observed deaths.

TABLE 4
Mortality From Cancer of the Intestine (Excludes Rectum) and Cancer of the Bladder by Time Since First Employment (Latency) in Perchloroethylene Shops*

Latency (yr)	MN of Intestine Observed/Expected (SMR)	MN of Bladder Observed/Expected (SMR)
<10	0/1.8 —	0/0.4 —
10-19	6/3.9 (154)	0/0.9 —
20-29	10/4.9 (204)	5/1.1 (455)
≥30	0/1.2 —	3/0.3 (1,000)
Total	16/11.8 (136)	8/2.7 (296)

* Abbreviations used are: MN, malignant neoplasms; SMR, standardized mortality ratio.

TABLE 2
Standardized Mortality Rates for Selected Causes by Race and Sex Groups of Dry Cleaner Workers

Cause of Death	White Males	White Females	Nonwhite Males	Nonwhite Females
All causes	85	96	88	75
All malignant neoplasms	107	107	145*	110
MN of intestine	66	136	313	111
MN of pancreas	111	167	143	286
MN of kidney	143	333	—†	333
MN of bladder	273	400	500*	—
MN of breast	—	89	—	88
MN of cervix	—	188	—	206
Accidents	—	—	36*	—

* $P < .05$.

† — = no observed deaths.

TABLE 3
Mortality From Cancer of the Testes (Except Rectum) and Cancer of the Bladder by Length of Employment in Perchloroethylene Shops*

Length of Employment (yr)	MN of Testes Observed/Expected (SMR)	MN of Bladder Observed/Expected (SMR)
1-4	6/8.1 (98)	1/1.4 (71)
5-9	5/3.1 (161)	4/0.7 (571)
10-14	5/1.7 (294)	2/0.4 (500)
≥15	0/0.9 —	1/0.2 (500)
Total	16/11.8 (136)	8/2.7 (296)

* Abbreviations used are: MN, malignant neoplasm; SMR, standardized mortality ratio.

these were generally based on small numbers of observed and expected deaths. Kidney cancer was also elevated in three out of the four race/sex groups and two out of the four unions. It is interesting that both kidney and bladder cancer were found in excess. It can only be speculated that these two cancer sites are related to a common etiology. In studies of cigarette smokers,⁹ and of workers exposed to benzidine⁹, both sites were elevated.

When mortality from bladder cancer was examined by latency and exposure (based on employment in PCE facilities) a pattern consistent with an occupational etiology was found. The increased risk for bladder cancer mortality occurred after 20 years of latency which is similar to other studies of known bladder carcinogens.¹⁰

In this study the calculation of both exposure and latency associated with PCE are only estimates, because it was assumed that petroleum solvents were used during time periods of unknown solvent use and these time periods were not used in calculating exposure or latency. For some plants this assumption may be incorrect and PCE rather than petroleum solvents could have been used. This would tend to increase latency and exposure for the cohort. However, the bias that this creates depends on whether the bladder cancer deaths are affected more or less than others in the cohort.

In the analysis of workers employed only in shops where PCE was the primary solvent, there was no excess risk in mortality from bladder or kidney cancer. Therefore, the excess risk occurred in workers with a potential for mixed exposures to PCE and petroleum solvents. This finding does not preclude PCE as the exposure associated with the excess in urinary tract cancer but certainly weakens the possibility of an association. In addition, there is experimental evidence that kidney cancer may be related to exposure from petroleum solvents. Recently, in a study by Kitchen,¹¹ vaporized, unleaded gasoline induced kidney cancer in exposed Fisher 344/N rats. The chemical structure and toxicologic properties of Stoddard solvent, the petroleum solvent used in dry cleaning, is similar to those of gasoline.¹²

Cigarette smoking has also been associated with an excess risk of developing bladder cancer. Most studies have shown a twofold to fourfold excess risk of bladder cancer in male smokers compared to that in non-smokers.¹³ The role of cigarette smoking in the excess risks observed in this study cannot be determined quantitatively because no data were available on the smoking

habits of the study cohort. However, according to Axelson,¹³ smoking is a weak confounder unless it is strongly associated with the disease and unless smoking habits between exposed and nonexposed workers differ drastically. The possible effects from smoking on the risk for bladder cancer in this population were calculated based on the method described by Axelson.¹³ Based on these calculations, it can be concluded that smoking cannot account for the threefold excess seen in this cohort. In fact, if 100% of the population were heavy smokers, this would account for only a 56% increase in the risk.

The most striking finding in the study was the overwhelming deficit in mortality due to accidents. This may be due to the demographics of the study population, which is primarily lower socioeconomic and inner city (Chicago, New York City, Detroit, and Oakland, California) workers. Therefore, it is possible that these workers did not own automobiles and relied heavily on inner city transportation systems which would account for the deficit in transportation accidents.

The two deaths from renal calculi, both of which were found in the subcohort of workers employed only in PCE shops was an unexpected finding that may have been a chance occurrence. However, it is possible that exposure to PCE could be related to this finding. Although we know of no reports describing nephrotoxicity in humans exposed to PCE, renal effects have been observed in experimental animal studies.^{2,8} In addition, there have been reports in the literature describing the occurrence of urinary calculi due to chemical exposure. A person developed renal tubular acidosis and urinary calculi after persistent toluene sniffing,¹⁴ and ethylene glycol has been associated with the production of calcium oxalate in renal tubules leading to formation of calculi in rats.¹⁵ The role PCE might play in these types of mechanisms which lead to urinary calculi is unknown.

Several other epidemiologic studies of dry cleaner and laundry workers have been conducted. In a study by Blair et al¹⁶ a proportionate mortality ratio (PMR) analysis was performed on 330 death certificates obtained from two local laundry and dry cleaning unions. These deaths only represented a sample of the total number of deaths that occurred between 1957 and 1977 among the union members. The sample of deaths was not necessarily a probabilistic sample but included deaths that had been identified by the researchers. Workers were included regardless of the solvent used in the dry cleaning shop which employed the worker; therefore, it was not specifically a study of PCE expo-

sure. The risk of mortality from all cancers combined was found to be higher than expected (87 observed v 67.9 expected; $P < .05$). Among the cancer deaths, lung, cervix uteri, and skin cancers were elevated at a statistically significant level. Other malignant neoplasms found in excess were intestine, liver and leukemia. Risks for bladder, kidney and pancreatic cancer were not elevated, and breast cancer risk was lower than expected.

Katz¹⁷ studied the mortality of female laundry and dry cleaner workers in Wisconsin by identifying 671 deceased workers from the occupational statement listed on death certificates. He tested for associations between occupation and cause of death by calculating specific PMRs for 25 causes of death. As in Blair's study the workers did not necessarily work at shops using PCE. In contrast to Blair's study, the risk of death for all cancer, liver cancer, colon cancer, lung cancer and leukemia was not elevated. Statistically significant excess risks were found for cervix uteri, genital (unspecified), and kidney cancer (7 observed v 2.7 expected). Bladder cancer risk was also elevated (5 observed v 2.6 expected). Breast cancer risk was less than expected.

A study using similar methods was conducted by Duh and Asai¹⁸ in Oklahoma, where deaths from 440 laundry and dry cleaner workers were analyzed. Again, no identification of solvent use was attempted; however, the use of petroleum solvents is more common among the dry cleaning shops in Oklahoma where more than 50% of the shops use this solvent, whereas the remaining use PCE. Duh and Asai found a statistically significant excess risk for lung and kidney cancer. Other elevated risks were found for cervix uteri, other female genital organs, and skin cancer. The risks for liver, pancreas, and bladder cancer were lower than expected.

The mortality risks for these previous studies of dry cleaner workers along with those of the present study have been summarized in Table 6. There were several causes that exhibited consistent excess risks, including cancer of the cervix uteri and kidney. A deficit was observed in all studies for breast cancer. As discussed earlier, the results for cervix uteri and breast are

probably related to the socioeconomic status of these occupational groups.

Conclusion

The excess risk for urinary tract cancer in this study was somewhat unexpected. Because multiple causes of death were examined and this cause of death was not part of an a priori hypothesis, the finding may be due to chance. However, it was the only cause found to have a statistically significant excess in mortality and the excess risk was found in three out of the four race/sex groups and in three out of the four unions. The magnitude of the SMR and the pattern by latency and exposure to PCE was consistent with an occupational carcinogen. However, because of the limitation in the records used to identify the cohort, it is not possible to analyze the data by specific job or estimate of exposure to PCE. Therefore, a dose-response analysis for urinary tract cancer, other than by length of employment, is not possible. Even the analysis by length of employment is limited as a surrogate of exposure because of missing data on solvent use. Inasmuch as many of the workers could have had confounding exposures to petroleum solvents in dry cleaning shops, those with employment in PCE shops only were analyzed separately. In this "PCE only" subcohort there was no excess risk for urinary tract cancer. Therefore, the confounding exposure to petroleum solvents complicates any conclusions regarding the association between PCE exposure and cancer of the urinary tract.

Acknowledgments

We thank the staff of the Stanford Research Institute (SRI) International for obtaining, coding, and editing much of the data used in the study, and the clerical staff of the National Institute for Occupational Safety and Health, Industrywide Studies Branch under the direction of Edith Dodd, Gerardo Santiago, and Pauline Blashaw, and Janet Grayson for preparation of the manuscript. We also thank Dr William Halperin for his comments, the participating unions and the International Fabricare Institute for their cooperation.

References

1. National Occupational Hazard Survey, vol 3: Survey Analysis and Supplemental Tables. Dept. of Health, Education and Welfare National Institute for Occupational Safety and Health, publication No 78-114, 1977 (with data base update as of Aug 6, 1980).
2. Biometry of Tetrachloroethylene for Possible Carcinogenicity Carcinogenesis Technical Report Series, No. 13, Dept. of Health, Education and Welfare, National Cancer Institute, publication No. (NCH) 77-50, 1977.
3. National Toxicology Program Technical Report on the Toxicology and Carcinogenic Studies of Tetrachloroethylene (Perchloroethylene) in F344/N Rats and B6C3F1 Mice. National Institutes of Health publication No. 88-2667, August 1988 (heard draft).
4. Ludwig HL, Meister MV, Roberts DR, et al: Worker exposure to perchloroethylene in the commercial dry cleaning industry. *Am Ind Hyg Assoc J* 1988;49:600-608.
5. Waxweiler RJ, Benumet JJ, Henry JA, et al: A modified U.S.

TABLE 6
Comparison of Cause-Specific Risks Among Mortality Studies of Dry Cleaner (and Laundry) Workers*

Cause of Death	Blair et al ¹⁶ (PMR)	Katz ¹⁷ (PMR)	Duh and Asai ¹⁸ (SMOR)	Brown ¹ (SMR)
All cancer	128†	96	90	116
Intestine	152	103	60	136
Liver	235	89	50	—‡
Pancreas	129	117	50	172
Lung	170†	98	170	114
Skin	429†	207	150	—
Breast	69	72	10	87
Cervix uteri	208†	195†	130	196
Bladder	83	189	40	296†
Kidney	200	257	380	200
Leukemia	227	67	—	—

* Abbreviations used are: PMR, proportionate mortality ratio; SMOR, standardized mortality odds ratio; SMR, standardized mortality ratio.

† $P < .05$.

‡ — = not calculable—no observed deaths.

table analysis system for cohort studies *J Occup Med* 1983;25:113-124.

6. Breslow NE, Lubin JH, Langholz B: Multiplicative models and cohort analysis. *J Am Stat Assoc* 1983;78:1-12.

7. Hoover R, Mason TJ, Mabey FW, et al: Geographic patterns of cancer mortality in the United States. In Fraumeni JF (ed): *Cancer as High Risk of Cancer: An Approach to Cancer Etiology and Control*. New York: Academic Press, 1973, pp 343-360.

8. Hammond EC: Smoking in relation to the death rates of one-million men and women. In Haccot W (ed): *Epidemiological Approaches to the Study of Cancer and Other Chronic Diseases*. US Dept of Health, Education and Welfare, Public Health Service, National Cancer Institute Monograph 19, 1973, pp 127-204.

9. Zaveri MB, Hoeggy U, Bingham E: Benzidine exposure as a cause of bladder tumors. *Arch Environ Health* 1973;27:1-7.

10. Maccowen GM, Elliott EA: Bladder cancer epidemiology. *Epidemiol Rev* 1981;3:203-229.

11. Kitchin DN: Neoplastic renal effects of unaltered gasoline in Fischer 344 rats. In Melikian MA (ed): *Renal Effects of Petroleum*

Hydrocarbons: Advances in Modern Environmental Toxicology. Princeton, NJ: Princeton Scientific Publishers Inc, 1984, vol 7, pp 45-71.

12. Sandmeyer EE: Aromatic hydrocarbons. In Clayton GD (ed): *Patty's Industrial Hygiene and Toxicology*, ed 3. New York: John Wiley and Sons, 1981, vol 2B, pp 3253-3453.

13. Aroson O: Aspects of confounding in occupational health epidemiology (letter). *Scand J Work Environ Health* 1978;4:98-102.

14. Kroger RM, Moore RJ, Lehman TE, et al: Recurrent urinary calculi associated with talcose mulling. *J Urol* 1980;124:89-91.

15. Williams EE, Smith LH: Disorders of excrete metabolism. *Am J Med* 1968;45:715-735.

16. Blair A, Demuelle P, Grauman D: Causes of death among laundry and dry cleaning workers. *Am J Public Health* 1968;58:608-611.

17. Katz RM, Jowers D: Female laundry and dry cleaning workers in Wisconsin: A mortality analysis. *Am J Public Health* 1981;71:305-307.

18. Duh RW, Axel NR: Mortality among laundry and dry cleaning workers in Oklahoma. *Am J Public Health* 1984;74:1278-1280.

The Artist's Dream

By artist I mean . . . everyone who has tried to create something which was not here before him, with no other tools and material than the uncommercial ones of the human spirit: who has tried to carve, no matter how crudely, on the wall of that final oblivion, in the tongue of the human spirit, "Elroy was here."

That is primarily . . . all . . . we ever really tried to do. And I believe we will all agree that we failed. That what we made never quite matched and never will match the shape, the dream of perfection which we inherited and which drove us and will continue to drive us, even after each failure, until anguish frees us and the hand falls still at last.

Maybe it's just as well that we are doomed to fail, since, as long as we do fail and the hand continues to hold blood, we will try again: where, if we ever did attain the dream, reach the shape, scale that ultimate peak of perfection, nothing would remain but to jump off the other side of it into suicide. . . .

—Comments by William Faulkner upon receipt of National Book Award for fiction in 1955. From "Three Cheers for Good Marks: Writers on Their Prizes" in *The New York Times Book Review*, Nov 16, 1986



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

March 9, 1988

SAB-EHC-88-011

Hon. Lee M. Thomas
Administrator
U.S. Environmental Protection
Agency
401 M Street, SW
Washington, D.C. 20460

OFFICE OF
THE ADMINISTRATOR

Dear Mr. Thomas:

Thank you for your thoughtful response of August 3 to the Science Advisory Board's review of scientific evidence associated with exposure to perchloroethylene. In your letter you asked the Board to provide further scientific advice on three issues that will subsequently bear on your risk management decision for this compound. The Board appreciates this opportunity for further scientific dialogue on these issues and hopes that its views in this letter can better promote consensus on the scientific issues under review.

As noted in your letter, the assessment of the scientific evidence from experimental animal studies centers on the relative significance for humans of the production of rat kidney and mouse liver tumors. This question is applicable to a broad range of chlorinated hydrocarbon compounds—including dichloromethane, para-dichlorobenzene, trichloromethane, and trichloroethylene—which produce tumors of the rat kidney and mouse liver under some experimental conditions. While recognizing the implications of such issues to these and other compounds, this letter is directed specifically to an assessment of perchloroethylene.

In responding to your letter, the Board's Environmental Health Committee and its Halogenated Organics Subcommittee organized a scientific workshop on August 12, 1987 to explore these and other issues with leading researchers in the field, EPA staff and members of the public. An agenda of the workshop is attached. The Board has utilized the information obtained in this workshop, and the discussions among Committee and Subcommittee members, to respond to your August 3 letter and also to advise the Agency on health effects evaluated in its Draft Health Assessment Document Addenda for Dichloromethane and Trichloroethylene. The Board's findings and recommendations on these latter two compounds will be transmitted to you in separate letters. Our response to your specific questions follows.

Question 1: Assuming that not all animal tumors are of equal significance to evaluating human hazard, what is the Science Advisory Board's current consensus position, based on scientific evidence or professional judgment, of the relative significance of male rat kidney or mouse hepatocellular tumors for human risk assessment?

SAB Response: In general, the Board's consensus conclusion on the significance of male rat kidney tumors stems from recent research (not yet published, but in

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press) that indicates that for many halogenated organics, probably including perchloroethylene, the mechanism producing these type of tumors is probably not operative in humans and, therefore, may not be relevant for human risk assessment. This mechanism involves the metabolism of the compound in the liver and the binding of a protein (alpha-2u-globulin) with the metabolite as a conjugate molecule. This molecule is filtered and accumulates in the kidney. One hypothesis is that the conjugate is more difficult to metabolize than the alpha-2u-globulin alone. This protein then accumulates and is injurious to the cell. Repair is followed by a cancerous formation at the site in a low percentage of cases. From available scientific evidence, this mechanism appears to be unique to male rats.

Thus far, thirteen substances have been demonstrated to produce renal tumors in male rats through this mechanism including perchloroethylene, para-dichlorobenzene and unleaded gasoline. Trichloroethylene, on the other hand, appears to produce renal tumors in male rats through a different (unknown) mechanism, thus creating important implications for human health risk assessment.

The Board's consensus on the significance of mouse liver tumors is 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. It concludes 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 tri-halomethyl radical formation), the one most promising for immediate application to risk assessment is characterized by proliferation of peroxisomes, an intracellular organelle, in the liver.

Peroxisome proliferation may be important for compounds such as perchloroethylene, but liver tumors observed after exposure to chlorinated solvents may involve different mechanisms. The importance of understanding the biological mechanisms is that they may provide a basis other than the bioassay statistical analysis for low-dose risk estimation. A plausible mechanism (peroxisome proliferation or something else) may imply low-dose nonlinearity for some substances that induce mouse liver tumors. However, different (presumably linear) mechanisms may operate for other substances, and these mechanisms may be consistent with linearity at low doses or a linear relationship to dose. These distinctions in low dose risk estimation should be explicitly included in the quantitative estimate of human risk.

Several substances that induce peroxisome proliferation in rodent livers, such as hypolipidemic drugs and the plasticizer di-ethylhexylphthalate (DEHP), also produce liver tumors in rodents. In summary, however, a causal relationship for this mechanism is plausible but unproven.

Some scientists have reported the detection of oncogenes after administration of presumably non-genotoxic agents.¹

¹ Steven H. Reynolds, Shari J. Stowers, Rachel M. Patterson, Robert R. Maronpot, Stuart A. Aaronson, Marshall W. Anderson, "Activated Oncogenes in B6C3F1 Mouse Liver Tumors: Implications for Risk Assessment," Science Vol. 237 (September 11, 1987), pp. 1309-1316.

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Also, as you are aware, our increasing knowledge of the role of mechanisms of promotion (later events in the carcinogenic process) may well clarify our understanding of cancer induction; certainly this is the case with dioxin and may relate to the halogenated hydrocarbons.

Question 2: What is the Board's view of the approach taken by EPA in using its guidelines to infer human carcinogenic potential from the total body of scientific evidence on perchloroethylene?

SAB Response: The issues regarding the application of the risk assessment guidelines appear not to represent disagreement among scientists about scientific evidence but, rather, the consequence of attempting to fit the weights of evidence into necessarily arbitrary categories of risk. Since the weights of evidence, and uncertainties associated with such evidence, for perchloroethylene and other compounds fall within a range of scientifically defensible choices, it may not be possible, in some instances, to fit them neatly into only one risk category. Moreover, the more incomplete the data, the less precision one can expect in classifying a compound within EPA's cancer guidelines. In addition, the type of evidence that places a compound in a particular category may vary considerably from substance to substance within that category. For perchloroethylene, as with trichloroethylene, the Science Advisory Board concludes that the overall weight of evidence lies on the continuum between the categories B₂ and C of EPA's risk assessment guidelines for cancer.

As perchloroethylene illustrates, the distinction between the B₂ and C categories can be an arbitrary distinction on a continuum of weight of evidence. The "black-white interpretation" that you referred to in your letter is indeed troubling. From a scientific point of view, it seems inappropriate for EPA and other agencies to regulate substances that are classified B₂ and not to consider regulation of compounds classified as C, regardless of the level of human exposure. In the case of B₂, B₁ or even A categorized compounds where exposure levels are low, EPA may, with scientific justification, decline to regulate because the potential health effects appear to be trivial in magnitude. 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). Indoor exposure to perchloroethylene, such as might be found in dry cleaning establishments not using the equivalent of good industrial hygiene practices, could merit action under this criterion. So might high levels of exposure to other solvents, pesticides or industrial chemicals that have been considered by the public as "safe" in the absence of sufficient evidence of carcinogenicity in animals. In many instances, this appearance of safety results from not yet having the results from well-designed bioassays such as those conducted by the National Toxicology Program.

Finally, you noted the evaluation of perchloroethylene by the International Agency for Research on Cancer (IARC) as an example of evolving terminology for classifying potential carcinogens. In general, the Board believes that public understanding of complex scientific issues is enhanced when scientists and regulators can speak with a common voice. In view of its own experience of using the cancer risk assessment guidelines and, in particular, having to address the issue of the scientific uncertainty that exists among and within guideline categories, EPA should re-evaluate its labeling system and methods for characterizing uncertainty. It should also review whether to be more consistent with IARC's terminology.

Question 3: Is there research underway or anticipated that will clarify these rodent tumor responses and their relationship to human health risk assessment? What additional research should be undertaken?

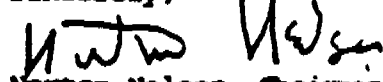
SAB Response: Current research undertaken in various laboratories, including the National Institutes of Health, can reduce some of the uncertainties associated with rodent tumor responses. Research results and hypotheses presented at the Board's August 12 workshop has served to clarify our understanding of male rat kidney tumors and their significance for human risk assessment. In addition, the Reynolds et. al. paper supplements our knowledge of activated oncogenes in mouse livers tumors.

Several research efforts should be initiated to further narrow scientific uncertainty for perchloroethylene and structurally related compounds. These include:

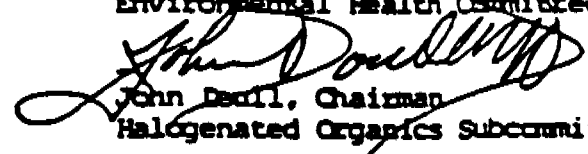
- o Validation of mechanistic models for the rat kidney and mouse liver tumors through experimentation with selected known carcinogens and non-carcinogens.
- o Development of improved methods for assessing low-dose response to environmental pollutants that induce peroxisome proliferation.

Once again, we are pleased to have this opportunity to present the views of the Science Advisory Board on these important scientific issues. We hope that the consensus stated above assists you in making the difficult risk management decisions on perchloroethylene and other compounds.

Sincerely,


Norton Nelson, Chairman
Executive Committee


Richard A. Griesemer, Chairman
Environmental Health Committee


John Deull, Chairman
Halogenated Organics Subcommittee

U.S. ENVIRONMENTAL PROTECTION AGENCY
SCIENCE ADVISORY BOARD
ENVIRONMENTAL HEALTH COMMITTEE/HALOGENATED ORGANICS
SUBCOMMITTEE (COMBINED ROSTER)

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EXECUTIVE SECRETARY

Dr. C. Richard Cothorn, Executive Secretary, Science Advisory Board
[A-101F] U.S. Environmental Protection Agency, Washington, D.C. 20460

NOTE: This combined roster only include those individuals attending the meeting.

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Young Scientists Award Lecture 1984:

**Species Differences in Carcinogenicity and Peroxisome Proliferation
due to Trichloroethylene: A Biochemical Human Hazard Assessment**

C. R. Elcombe

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Abstract. Trichloroethylene (TRI) administered to mice by gavage for 10 consecutive days at doses of 50–2000 mg/kg body weight elicited dose-dependent increases (up to 700% of control values) of hepatic cyanide insensitive palmitoyl CoA oxidation (a marker of peroxisomal β -oxidation). No effect was seen on catalase; the other peroxisomal marker examined. Similar experiments with rats demonstrated no effect of TRI on either cyanide insensitive palmitoyl CoA oxidation or catalase. A major metabolite of TRI, trichloroacetic acid (TCA) when administered by gavage for 10 consecutive days at doses of 10–200 mg/kg body weight, stimulated hepatic cyanide insensitive palmitoyl CoA oxidation in both mice (up to 500% of control) and rats (up to 650% of control). Again, no effect upon catalase activity was apparent. The kinetics of biotransformation of TRI to TCA in isolated hepatocytes was markedly species dependent. The 'intrinsic clearance' values (V_{max}/K_m) for TRI in mouse, rat and human hepatocytes were 3.8×10^{-6} , 1.2×10^{-7} and 3.25×10^{-8} L/min/ 10^6 cells respectively. TCA induced peroxisomal β -oxidation in mouse and rat hepatocytes, but had no effect upon this enzyme activity in cultured human hepatocytes. It is postulated that the species difference in hepatocarcinogenicity of TRI (mouse positive; rat negative) is due to species differences in peroxisome proliferation which in turn is a result of differences in the rate of formation of TCA from TRI. On this basis it is proposed that TRI presents no significant human hepatocarcinogenic hazard since, (1) human hepatocytes produced TCA at a rate even lower than that of the rat, and (2) TCA was not a peroxisome proliferator in human hepatocytes.

Key words: Trichloroethylene – Peroxisome Proliferation – Species Differences – Hepatocellular Carcinoma

Introduction

Trichloroethylene (TRI), administered orally at high doses for 18 months has been shown to increase the incidence of hepatocellular carcinoma in B6C3F₁ mice but not Osborne-Mendel rats (NCL 1976). The interpretation of these studies has been confounded due to the presence of epoxide stabilizers in the TRI. However more recent studies have demonstrated that pure TRI also causes hepatocellular carcinoma in B6C3F₁ mice (NTP, 1983) and Alderley Park (Swiss) mice (Elcombe and Pratt, unpublished data). Furthermore, no increase in the incidence of hepatocellular carcinoma was observed in Fisher 344 rats administered pure TRI (NTP, 1983).

TRI has been extensively examined for mutagenic potential, but many studies were bedeviled by the presence of mutagenic epoxide stabilizers. However, in general TRI has been found to be only 'marginally' mutagenic or non-mutagenic (Greim et al., 1975; Simmon et al., 1977; Bronzetti et al., 1978; Waskell, 1978; Bartsch et al., 1979; Slacik-Erben et al., 1980).

Covalent binding of trichloroethylene or its metabolites to protein, RNA and DNA has been illustrated *in vitro* (Van Duuren and Banerjee, 1976; Bolt et al., 1977; Bolt and Fülser, 1977; Uehleke and Poplawski-Tabarelli, 1977; Banerjee and Van Duuren, 1978). However, *in vivo*, only extremely low (indistinguishable from protein binding) or zero binding of TRI metabolites to DNA has been reported (Parchman and Magee, 1982; Stott et al., 1982).

Hence, TRI does not appear to be a classical genotoxic carcinogen, and probably exerts its carcinogenic potential via an epigenetic mechanism. Indeed, Schumann et al. (1980) and Stott et al. (1982) have speculated that TRI manifests its hepatocarcinogenicity due to sustained cytotoxicity.

Other epigenetic mechanisms of chemical carcinogenicity have been discussed (Thorpe et al., 1982), one such mechanism being the phenomenon of peroxisome proliferation. Several nonmutagenic chemicals which are hepatocarcinogenic to rodents have been shown to elicit hepatic peroxisome proliferation (Hess et al., 1965; Svoboda et al., 1967; Moody and Reddy, 1975; Reddy et al., 1980). It has been proposed that a causal relationship exists between peroxisome proliferation and the development of hepatocellular carcinoma (Reddy et al., 1980). The mechanism(s) involved in neoplastic transformation after the administration of peroxisome proliferators is unclear, however Reddy et al. (1980; 1982) have postulated the involvement of reactive O₂ species.

Preliminary studies in this laboratory have shown TRI to elicit hepatic peroxisome proliferation in mice but not rats. Hence, the present study was initiated to (1) investigate the possible involvement of peroxisome proliferation in trichloroethylene carcinogenicity, (2) to explain species differences in such carcinogenicity and (3) attempt to obtain a human hazard assessment using isolated and cultured human hepatocytes.

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Methods

Reagents

Trichloroethylene (>99.9% by gas liquid chromatography, containing 0.02% w/w triethylamine stabilizer) was obtained from Imperial Chemical Industries PLC, Mond Division, Cheshire, UK. All biochemicals were obtained from Sigma London Chemical Company (Dorset, UK). Trichloroacetic acid (TCA) and other chemicals were purchased from BDH Chemicals Ltd (Liverpool, UK). Cell culture materials were obtained from Flow Laboratories (Irvine, Scotland, UK).

Animals

Male Alderley Park rats (Wistar derived) and male Alderley Park mice (Swiss) weighed 180–220 g and 25–30 g respectively at the start of the studies.

The animals were housed in suspended stainless steel wire mesh cages and fed throughout the studies with PCD diet (Special Diet Services Ltd, Witham, Essex, UK) and allowed tap water ad libitum. The animals were exposed to a light/dark cycle of 12 hr (0600–1800 light).

In vivo studies

Animals were administered trichloroethylene (50–2000 mg/kg body weight) or trichloroacetic acid (10–200 mg/kg) dissolved in corn oil, by gavage daily for 10 consecutive days. Control animals received an appropriate volume of corn oil vehicle alone (10 ml/kg body weight).

The animals were killed by cervical dislocation 24 hr following the final dose of TRL, TCA or corn oil, and the livers rapidly excised and weighed. The livers were homogenized in 4 volumes 20 mM TRIS HCl (pH 7.4) containing 5.4 mM EDTA and 250 mM sucrose (SET buffer). The homogenate was centrifuged at 600 g (average) for 5 min at 4°C to remove connective tissue, intact cells and nuclei. The resultant supernatant was centrifuged at 15,000 g (average) for 15 min at 4°C to sediment a heavy pellet consisting of peroxisomes, mitochondria and lysosomes. This pellet was suspended in SET buffer to a final protein concentration of 20–30 mg/ml. Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin standards.

Catalase activity and cyanide insensitive palmitoyl CoA oxidation (a peroxisomal β -oxidation marker) were determined spectrophotometrically in the resuspended 15,000 g pellets by the methods of Beers and Sizer (1952) and Bronfman et al. (1979) respectively.

Statistics

All values are expressed as Mean $\pm$ SEM. Statistical significance was determined using Student's t-test (two-tailed), a level of $p < 0.05$ being considered as significant.

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In vitro studies

Cell culture. Hepatocytes were isolated from rats and mice by a two step in situ perfusion technique as described previously (Mitchell et al., 1984). The isolated cells were suspended in Liebowitz L15 medium containing foetal bovine serum (8.3%), tryptose phosphate broth (8.3%), penicillin G (41.3 IU/ml), streptomycin sulphate (8.2 µg/ml), glutamine (241 µg/ml), insulin (10^{-6} M) and hydrocortisone (10^{-8} M). The viability of the hepatocytes (>95%) was determined by trypan blue exclusion. 25 cm² Falcon tissue culture flasks were seeded with 2×10^6 viable hepatocytes contained in 4 ml of culture medium. The flasks were incubated at 37°C in air. 4, 24, 48 and 72 hr after seeding, the spent medium and any detached cells were aspirated and fresh medium applied.

Human liver was obtained from brain-dead renal transplant donors after compliance with ethical and legal requirements. The liver was sliced (0.5 mm slices) by hand and digested in Hank's buffer containing 0.3% (w/v) dispase, 0.1% (w/v) collagenase, 0.1% (w/v) hyaluronidase, 0.05% (w/v) deoxyribonuclease and 50 µg/ml gentamicin at 37°C for 1 hr. Cells were recovered by sieving through a 120 µ bolting cloth and centrifugation for 2 min at 100 g. Cells were then treated as for rat and mouse hepatocytes except ascorbic acid (50 mg/l) was added to the culture medium.

Trichloroacetic acid, dissolved in N,N-dimethylformamide was added to the monolayer cultures at each 24 hour medium change. The amount of dimethylformamide never exceeded 10 µl per flask (4 ml) and this concentration produced no obvious cytotoxicity and had no effect upon the parameters measured.

96 hours after seeding, the hepatocytes were harvested. The medium was discarded and the cells washed in 2 ml SET buffer. The cells were removed from the flask by scraping with a rubber policeman into 1 ml of SET buffer. The cells were disrupted by sonication and the resultant homogenate used for the determination of protein content and cyanide insensitive palmitoyl CoA oxidation.

Metabolism of TRI by freshly isolated hepatocytes

Rat, mouse and human hepatocytes were isolated as described earlier, and suspended in the complete Liebowitz L15 culture medium. TRI (0.02-2 mM) was added and the cells incubated at 37°C in sealed 25 ml Pierce 'Reacti-flasks' for periods up to 1 hr. Formation of product was linear over the time periods utilized. Initial rates for TCA formation were calculated and kinetic parameters estimated graphically using Woolf plots (S/V versus S).

Trichloroacetic acid was extracted from acidified incubation mixtures using diethylether and derivitized with diazomethane. Quantitation was by gas-liquid chromatography (Carbopak C SP1000 0.1% 80-100 mesh, 3 feet long, 145°C) with electron capture detection.

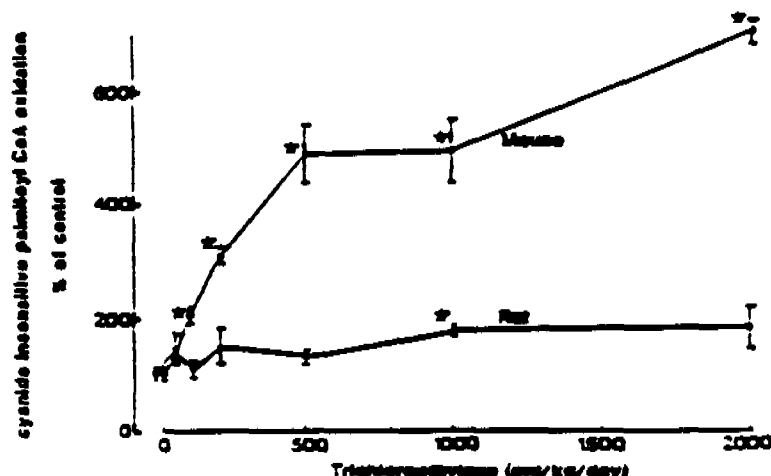


Fig. 1. Dose-dependent induction of peroxisomal cyanide insensitive palmitoyl CoA oxidation by trichloroethylene.

Animals were administered trichloroethylene (50–2000 mg/kg/day) by gavage for 10 consecutive days. Controls received corn oil vehicle alone. Values are Mean $\pm$ SEM ($n=4-5$). Control rates of cyanide insensitive palmitoyl CoA oxidation were: Rat, 7.70 ± 0.69 (5); Mouse, 9.34 ± 0.35 (5) nmol NAD⁺ reduced/min/mg protein. *Significantly different from respective control, $p < 0.05$.

Results

In vivo effects of trichloroethylene on hepatic peroxisomal enzyme activity

The administration of TRI to mice for 10 consecutive days resulted in a dose-related increase in hepatic peroxisomal β -oxidation (cyanide insensitive palmitoyl CoA oxidation), a 7-fold increase being observed at a dose of TRI of 2000 mg/kg/day. No significant alteration in peroxisomal β -oxidation was observed in rats after a similar treatment regimen (Fig. 1). Catalase, also a peroxisomal marker enzyme, was effectively unchanged in both species of animal (Table 1). Electron microscopy indicated an increased peroxisome volume density (expressed as % of cytoplasmic volume) in mice but not rats (Table 2).

In vivo effects of trichloroacetic acid on hepatic peroxisomal enzyme activity

Trichloroacetic acid (TCA) is a major metabolite of TRI. The administration of TCA to rats and mice led to dose-related increases in cyanide insensitive palmitoyl CoA oxidation in both species. At doses of 200 mg/kg/day for 10 days, 6.5-fold (rat) and 4.8-fold (mouse) increases in peroxisomal β -oxidation were observed (Fig. 2). TCA, in common with TRI, had little, if any, effect on hepatic catalase activity (Table 3). Once again peroxisome volume densities were increased concomitantly with β -oxidation activity (Table 4).

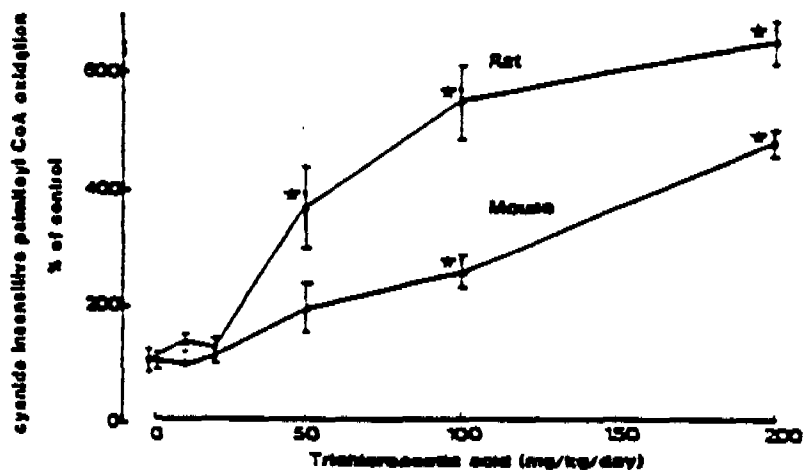


Fig. 2. Dose-dependent induction of peroxisomal cyanide insensitive palmitoyl CoA oxidation by trichloroacetic acid.

Animals were administered trichloroacetic acid (10–200 mg/kg/day) for 10 consecutive days. Controls received corn oil vehicle alone. Values are Mean $\pm$ SEM ($n=4-5$). Control rates of cyanide insensitive palmitoyl CoA oxidation were: Rat, 1.81 ± 0.82 (5); Mouse, 10.30 ± 1.37 (4) nmol NAD⁺ reduced/min/mg protein. *Significantly different from respective control, $p < 0.05$.

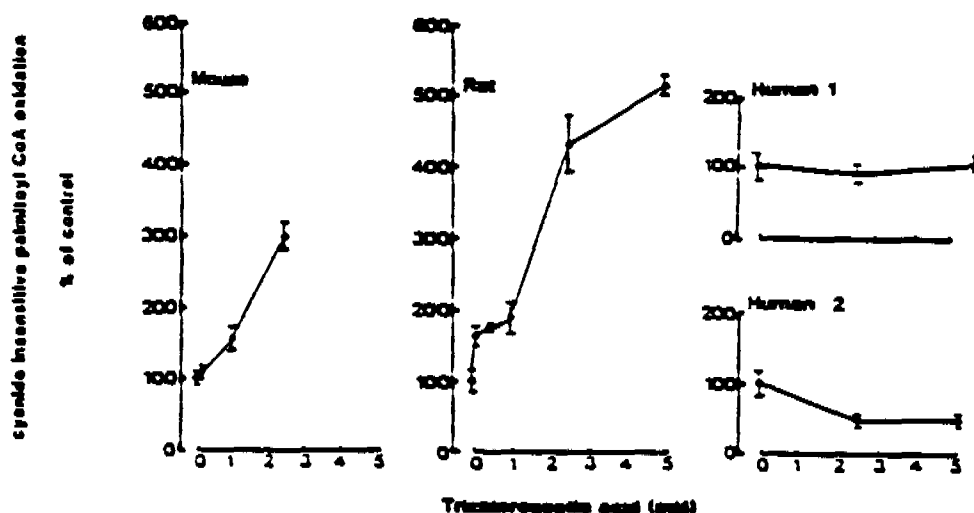


Fig. 3. Effect of trichloroacetic acid on peroxisomal β -oxidation activities of rat, mouse and human hepatocytes.

Cells were cultured as described in Methods, and exposed to various concentrations of trichloroacetic acid for 3 days. After this time peroxisomal β -oxidation was determined as cyanide insensitive palmitoyl CoA oxidation.

Values are Mean $\pm$ SEM ($n=4$ replicates) from typical rat and mouse hepatocyte preparations. For human hepatocytes values are Mean $\pm$ SEM ($n=3$ replicates) for 2 individual liver samples.

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Table 1. Effect of trichloroethylene on hepatic catalase activity in rats and mice

Dose of trichloroethylene (mg/kg/day)	Catalase ($K \cdot s^{-1} \cdot mg^{-1}$)	
	Mice	Rat
0	0.82 ± 0.05 (100)	0.96 ± 0.13 (100)
50	0.81 ± 0.03 (98)	0.98 ± 0.09 (102)
100	1.09 ± 0.12 (132)	1.11 ± 0.09 (115)
200	1.09 ± 0.21 (132)	1.28 ± 0.18 (133)
500	0.84 ± 0.19 (102)	0.82 ± 0.09 (85)
1000	0.75 ± 0.02 (91)	0.88 ± 0.03 (91)
2000	0.78 ± 0.03 (95)	0.82 ± 0.08 (85)

Animals were dosed with trichloroethylene (50–2000 mg/kg/day) for 10 consecutive days. Controls received corn oil vehicle alone.

Values are Mean $\pm$ SEM (n=5). Values in parentheses are % of control.

Table 2. Morphometric analysis of centrilobular hepatocytes of rats and mice administered trichloroethylene

Dose of trichloroethylene (mg/kg/day)	Per cent sinusoidal volume (% cytoplasmic volume)	
	Rat	Mice
0	1.8 ± 0.3	1.5 ± 0.2
500	ND	4.7 ± 1.25
1000	2.4 ± 0.5	19.6 ± 2.3
1500	2.1 ± 0.6	21.4 ± 3.6

Animals were administered trichloroethylene in corn oil for 10 consecutive days.

Values are Mean $\pm$ SEM (n=4–6). ND, not determined.

Table 3. Effect of trichloroacetic acid on hepatic catalase activity in rats and mice

Dose of trichloroacetic acid (mg/kg/day)	Catalase ($K \cdot s^{-1} \cdot mg^{-1}$)	
	Mice	Rat
0	0.69 ± 0.02 (100)	0.76 ± 0.03 (100)
10	0.71 ± 0.04 (102)	0.86 ± 0.04 (113)
20	0.66 ± 0.04 (95)	1.05 ± 0.14 (138)
50	0.58 ± 0.10 (84)	0.88 ± 0.12 (115)
100	0.71 ± 0.04 (102)	0.73 ± 0.06 (96)
200	1.05 ± 0.45 (152)	0.58 ± 0.04 (76)

Animals received 10–200 mg/kg/day of trichloroacetic acid for 10 consecutive days. Controls received corn oil vehicle alone.

Values are Mean $\pm$ SEM (n=4–5). Values in parentheses are % of control.

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Table 4. Morphometric analysis of centriobular hepatocytes of rats and mice administered trichloroacetic acid

Dose of trichloroacetic acid (mg/kg/day)	Peroxisome density volume (% cytoplasmic volume)	
	Rats	Mice
0	1.9 ± 0.5	1.0 ± 0.2
50	4.2 ± 0.9	2.9 ± 0.7
100	3.9 ± 0.5	10.0 ± 2.5
200	7.9 ± 1.2	8.9 ± 2.1

Animals were administered trichloroacetic acid in corn oil for 10 consecutive days. Values are Mean ± SEM (n = 3-5). ND, not determined.

Table 5. Kinetics of formation of trichloroacetic acid from trichloroethylene by isolated mouse, rat and human hepatocytes

Species	K _m (μM)	V _{max} (pmol/min/10 ⁶ cells)	V _{max} /K _m (L/min/10 ⁶ cells)
Mouse	28 ± 9	107 ± 18	3.8 × 10 ⁻⁶
Rat	151 ± 51	19 ± 7	1.2 × 10 ⁻⁷
Human	168 (170, 165)	5.6 (8.2; 2.9)	3.25 × 10 ⁻⁸ (4.8 × 10 ⁻⁸ ; 1.7 × 10 ⁻⁹)

Cells were incubated with various concentrations of trichloroethylene as described in Methods. Kinetic parameters were obtained graphically from Woolf plots. Values are Mean ± SEM (n = 4 for rats and mouse, n = 2 for human; individual values are shown in parentheses).

In vitro induction of peroxisomal β-oxidation

The exposure of cultured rat and mouse hepatocytes to TCA resulted in dose-related increases in cyanide insensitive palmitoyl CoA oxidation in both species. However, when human hepatocytes were examined no stimulation of peroxisomal β-oxidation was observed (Fig. 3). This lack of response is believed not to be indicative of lack of viability of the human cells, because cells from the same liver responded to phenobarbitone and β-naphthoflavone as inducers of mixed function oxidase activity (unpublished data).

Metabolism of trichloroethylene by isolated hepatocytes

The kinetics of biotransformation of TRI to TCA were examined using freshly isolated hepatocytes. K_m values for TCA formation were similar in rat and human cells. However a considerably lower K_m for the reaction was manifest in mouse hepatocytes (Table 5). V_{max} values were highest in mouse cells, followed by rat cells

and human cells. Derivation of 'intrinsic clearance' or 'capacity' factors (V_{max}/K_m) illustrated 30-fold and 3-fold differences in efficacy of TCA formation between mouse and rat hepatocytes and rat and human hepatocytes respectively.

Discussion

This work has shown trichloroethylene to stimulate hepatic peroxisomal β -oxidation (an H_2O_2 -generating oxidase) in mice but not rats. This increase in enzyme activity was accompanied by a parallel increase in the number of and the volume density of peroxisomes (expressed as % of cytoplasmic volume). No effect upon hepatic catalase was observed. Although these experiments were performed in Alderley Park rats and mice, similar data has been obtained utilizing B6C3F₁ mice and Osborne-Mendel rats; the strains used in the NCI cancer bioassay (unpublished data).

Reddy et al. (1980) have postulated a causal relationship between peroxisome proliferation and the hepatocarcinogenicity of several non-mutagenic chemicals to rodents. These authors suggest that increased peroxisomal β -oxidation of fatty acids leads to the formation of increased steady state concentrations of H_2O_2 (Mannaerts et al., 1979), which in turn could lead to increased peroxidative damage of cellular components including DNA. This in turn could lead to a mutational event and eventually cancer according to the classical somatic mutation hypothesis. The potential for 'reactive O_2 ' cytotoxicity and genotoxicity may also be increased due to the lack of a parallel increase in catalase, a major H_2O_2 -detoxifying enzyme. Indeed, evidence for peroxidative damage to the liver of rodents following the administration of peroxisome proliferators has been presented (Lalwani et al., 1981; Reddy et al., 1982). Also, in this laboratory, the administration of trichloroethylene (1000 mg/kg/day) for two months and longer to B6C3F₁ mice has resulted in the deposition of lipofuscin - an indicator of oxidative damage and ageing (Miquel et al., 1977).

If this hypothesis is correct the species difference in susceptibility to TRI-induced hepatocarcinogenesis may be due to the species difference in peroxisome proliferation.

The present study has identified TCA as a peroxisome proliferator in both mice and rats. TCA is a major metabolite of TRI (Bulter, 1949); so why is TRI a peroxisome proliferator in mice but not in rats?

Previous studies (Elcombe et al., 1982; Green, personal communication), involving the oral administration of TRI, have demonstrated linear kinetics for TCA formation in mice and saturation kinetics in rats. That is, as the dose of TRI is increased proportionally more TCA is produced in mice. However, in similar experiments with rats, one reaches a point where no more TCA is formed and the TRI is eliminated from the rat unchanged. Detailed kinetic studies have shown that the maximum tolerable dose of TCA able to be produced in rats, irrespective of the dose of TRI, is about 50 mg/kg. This value is noteworthy, since in the present study no peroxisomal enzyme induction was observed below doses of 50 mg/kg TCA.

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This suggests that rats produce insufficient TCA from TRI to elicit peroxisome proliferation, thereby explaining the lack of peroxisome proliferation following TRI administration to rats. Furthermore, if peroxisome proliferation is causally related to tumour development, these observations would offer a mechanistic explanation of the species difference in carcinogenicity of TRI. This hypothesis demands that TCA should elicit hepatocellular carcinoma in both rats and mice; accordingly a two year study of TCA administration to rats and mice has started.

How do these observations help with a possible human hazard assessment? Firstly, peroxisome proliferation and the concomitant increase in peroxisomal β -oxidation have been evoked as indicators of hepatocarcinogenicity and, secondly, it is postulated that the differences in peroxisome proliferation and carcinogenicity of TRI are due to differences in the ability of rats and mice to produce TCA.

These present studies on the *in vitro* metabolism of TRI to TCA have shown that mouse hepatocytes have a 30-fold greater propensity for TCA production than rat hepatocytes, which in turn are 3-times more active than human cells. Therefore, it would appear that, in human liver TCA would be produced in smaller quantities than in rat liver and hence in insufficient amounts to elicit peroxisome proliferation after exposure to TRI. Furthermore, the addition of TCA, the proximate peroxisome proliferator, to human cells did not result in increased peroxisomal β -oxidation. Hence an intrinsic species difference in biological response appears to exist between rodent and human hepatocytes.

In summary, it is postulated that the species difference in the hepatocarcinogenicity of TRI is due to species differences in peroxisome proliferation, which, in turn, is a result of differences in the rate of formation of TCA from TRI. On this basis it is suggested that TRI presents no significant hepatocarcinogenic hazard to man since (1) human hepatocytes produced TCA at a rate less than rat hepatocytes and (2) TCA was not a peroxisome proliferator in human hepatocytes. Indeed this assertion is substantiated by several epidemiological studies which have demonstrated no increased incidence of liver tumours in humans exposed to trichloroethylene (Axelson et al., 1978; Malek et al., 1979; Novotna et al., 1979; Paddle, 1983).

References

- Axelson O, Anderson K, Heynck C, Holmberg B, Molina MD, de Verdier A (1978) A cohort study on trichloroethylene exposure and cancer mortality. *J Occup Med* 20: 194-196.
- Banerjee S and Van Duuren BL (1978) Covalent binding of the carcinogen trichloroethylene to hepatic macromolecular proteins and to exogenous DNA *in vitro*. *Cancer Res* 38: 776-780.
- Baron H, Malaveille C, Baron A and Flaminio G (1979) Mutagenic and alkylating metabolism of halogenated ethylenes, chloroethylenes and dichloroethylenes produced by rodent or human liver tissues. *Arch Toxicol* 41: 249-277.
- Barr RF and Sizer JW (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 195: 133-140.
- Bolt HM and Filser JG (1977) Irreversible binding of chlorinated ethylenes to macromolecules. *Environ Health Perspect* 21: 107-112.
- Bolt HM, Buchter A, Wolowitz C, Gil DL and Bolt W (1977) Incubation of ¹⁴C-trichloroethylene vapour with rat liver microsomes: uptake of radioactivity and covalent protein binding of metabolites. *Int Arch Occup Environ Health* 39: 103-111.
- Brownman M, Insarom NC and Lapham F (1979) Fatty acid oxidation by human liver peroxisomes. *Biochem Biophys Res Comm* 88: 1030-1036.

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- Brouwer G, Zeiger E and Frenn D (1978) Genetic activity of trichloroethylene in yeast. *J Environ Pathol Toxicol* 1: 411-418.
- Buzler TC (1949) Metabolic transformation of trichloroethylene. *J Pharmacol Exptl Therap* 97: 34-42.
- Elcombe CR, Pratt LS and Green T (1982) The rate of trichloroacetic acid (TCA) formation determines the species difference in hepatic peroxisome proliferation due to trichloroethylene (TCE). *The Pharmacologist* 24: Abstract 432.
- Green H, Boush G, Radwan Z, Ruchart D and Henschler D (1975) Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxo-formation. *Biochem Pharmacol* 24: 2013-2017.
- Hess R, Scuderi W and Rimm W (1965) Nature of the hepatomegalic effect produced by ethyl-chloroform- α -isobutyrate in the rat. *Nature* 208: 856-858.
- Laiwan ND, Reddy MK, Qureshi SA and Reddy JK (1981) Development of hepatocellular carcinomas and increased peroxisomal fatty acid β -oxidation in rats fed [4-chloro-4-(2,3-xylylene)-2-pyridinylthiol] acetate and (Wy-14,643) in the semi-purified diet. *Carcinogenesis* 2: 645-650.
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265-275.
- Malek B, Krutarsova B and Radova O (1979) An epidemiological study of the hepatic tumour incidence in persons working with trichloroethylene II - The negative results of investigations among dry cleaning workers. *Pracevni Lekarske* 31: 124-126.
- Mannervik GP, DeBeer LJ, Thomas J and De Schapper PJ (1979) Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate treated rats. *J Biol Chem* 254: 4585-4593.
- Miquel J, Oro J, Benito, KG and Johnson JE (1977) Lipofuscin: Fine structural and biochemical studies. In: *Free Radicals in Biology*, Vol 1, pp 133-182 (Ed WA Pryor), Academic Press (New York).
- Mitchell AM, Bridges JW and Elcombe CR (1980) Factors influencing peroxisome proliferation in cultured rat hepatocytes. *Arch Toxicol* 55: 239-246.
- Moody DE and Reddy JK (1978) Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds. *Toxicol Appl Pharmacol* 45: 497-504.
- National Cancer Institute (1978) Carcinogenesis bioassay of trichloroethylene. CAS No 79-01-6, DHEW Publ No (NIH) 76-802.
- National Toxicology Program (1983) National Toxicology Program draft report abstracts on nine chemical carcinogenesis bioassays. *Chemical Regulation Reporter* 6: 767-768.
- Novotna E, David A, Malek B (1979) An epidemiological study of the hepatic tumour incidence in persons working with trichloroethylene I - The negative results of retrospective investigations in persons with primary liver carcinoma. *Pracevni Lekarske* 31: 121-123.
- Paddle GM (1983) Incidence of liver cancer and trichloroethylene manufacture: Joint study by industry and a cancer registry. *Brit Med J* 286: 346-347.
- Parchman LG and Magnus PN (1982) Metabolism of [14 C]trichloroethylene to 14 CO $_2$ and interaction of a metabolite with liver DNA in rats and mice. *J Toxicol Environ Health* 9: 797-813.
- Reddy JK, Azarnoff DL and Higgins CE (1980) Hypolipidemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 283: 397-398.
- Reddy JK, Laiwan ND, Reddy MK and Qureshi SA (1982) Extensive accumulation of autofluorescent lipofuscin in the liver during hepatocarcinogenesis by methyl clofibrate and other hypolipidemic peroxisome proliferators. *Cancer Res* 42: 259-266.
- Schumann AM, Quast JF and Watanabe PG (1980) The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity. *Toxicol Appl Pharmacol* 55: 207-219.
- Simmons VF, Kaubauer K and Turner RG (1977) Mutagenic activity of chemicals identified in drinking water. In: *Progress in Genetic Toxicology* (Eds D Scott, BA Bridges and FH Sobels), pp 249-258. Elsevier, North Holland, Amsterdam.
- Slank-Jensen R, Røe R, Frands G and Ubbels R (1980) Trichloroethylene vapours do not produce dominant lethal mutations in male mice. *Arch Toxicol* 45: 37-40.
- Stout WT, Quast JF and Watanabe PG (1982) The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. *Toxicol Appl Pharmacol* 62: 137-151.
- Svetovid DJ, Green H and Azarnoff DL (1967) Microbodies in experimentally induced units. *J Cell Biol* 35: 127-132.

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Uehida H and Popirowski-Tabaruli S (1977) Irreversible binding of ¹⁴C-labelled trichloroethylene to some liver constituents in vivo and in vitro. Arch Toxicol 37: 239-294.

Van Duuren BL and Banerjee S (1976) Covalent migration of metabolites of the carcinogen trichloroethylene in rat hepatic microsomes. Cancer Res 36: 2419-2422.

Wentzel L (1978) A study of the mutagenicity of anesthetics and their metabolites. Mutat Res 57: 141-151.

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The Role of Trichloroacetic Acid and Peroxisome Proliferation in the Differences in Carcinogenicity of Perchloroethylene in the Mouse and Rat

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The Role of Trichloroacetic Acid and Peroxisome Proliferation in the Differences in Carcinogenicity of Perchloroethylene in the Mouse and Rat. ODUM, J., GREEN, T., FOSTER, J. R., AND HEXT, P. M. (1988). *Toxicol. Appl. Pharmacol.* 92, 103-112. Fischer 344 rats and B6C3F1 mice of both sexes were exposed to 400 ppm perchloroethylene (PER) by inhalation, 6 hr/day for 14, 21, or 28 days or to 200 ppm for 28 days. Increased numbers of peroxisomes were seen under the electron microscope and increased peroxisomal cyanide-insensitive palmitoyl CoA oxidation was measured (3.6-fold increase in males and 2.1-fold increase in females) in the livers of mice exposed to PER. Hepatic catalase was not increased. Peroxisome proliferation was not observed in rat liver or in the kidneys of either species. Trichloroacetic acid (TCA), a known carcinogen and hepatic peroxisome proliferating agent, was found to be a major metabolite of PER. Blood levels of this metabolite measured in mice and rats during and for 48 hr after a single 6-hr exposure to 400 ppm PER showed that peak blood levels in mice were 13 times higher than those seen in rats. Comparison of areas under the curves over the time course of the experiment showed that mice were exposed to 6.7 times more TCA than rats. The difference in metabolism of PER to TCA in mice and rats leads to the species difference in hepatic peroxisome proliferation which is believed to be the basis of the species difference in hepatocarcinogenicity. Peroxisome proliferation does not appear to play a role in the apparent carcinogenicity of PER in the rat kidney. © 1988 Academic Press, Inc.

Perchloroethylene (PER) (1,1,2,2-tetrachloroethylene) is a volatile liquid which is used extensively in the dry cleaning industry and as a general degreasant in manufacturing industry.

A significant increase in hepatocellular carcinoma has been observed in male and female mice but not rats in two carcinogenicity bioassays of PER. In the first study (NCI, 1977) Osborne-Mendel rats and B6C3F1 mice received PER by gavage in corn oil at doses of approximately 500 or 1000 mg/kg. Both dose groups showed about a 50% incidence of hepatocellular carcinoma in mice. In the second study (Mennear *et al.*, 1986) Fi-

scher 344 rats and B6C3F1 mice were exposed to PER by inhalation (mice 100 or 200 ppm and rats 200 or 400 ppm 6 hr/day). Increased hepatic tumor incidence was again observed in mice, 50% in low and high dose males and 26 and 72% in low and high dose females. In the latter study a low incidence of kidney tubular adenocarcinoma was observed in male rats at the highest dose.

The species difference in hepatocarcinogenicity is similar to that seen with trichloroethylene (TRI) (NCI, 1976; NTP, 1983). TRI has been shown to induce peroxisome proliferation in mouse liver but not rat liver, after oral administration (Elcombe *et al.*, 1985). A causal relationship has been suggested between hepatic peroxisome proliferation and

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hepatocellular carcinoma in rodents (Reddy *et al.*, 1980) although no such relationship has yet been shown between renal peroxisome proliferation and renal tubular adenocarcinoma (Reddy *et al.*, 1975, 1982). The species difference in hepatic peroxisome proliferation elicited by TRI is believed to be the basis of the species difference in carcinogenicity (Elcombe *et al.*, 1985).

Trichloroacetic acid (TCA), a major metabolite of TRI (Green and Prout, 1985) has recently been shown to be carcinogenic in the B6C3F1 mouse (Herren-Freund *et al.*, 1986). TCA has also been shown to be responsible for peroxisome proliferation in TRI-dosed mice (Elcombe, 1985). Quantitative differences in the metabolism of TRI in rats and mice and hence in circulating levels of TCA (Prout *et al.*, 1985) may lead to the species difference in peroxisome proliferation and consequent carcinogenicity. TCA is reported to be a major metabolite of PER in mice and rats (Ylner, 1961; Daniel, 1963; Dekant *et al.*, 1985) and may therefore elicit the same response when animals are exposed to PER. In view of the lack of mutagenicity of PER (Greim *et al.*, 1975; Barsch *et al.*, 1979) this may be the basis for the species difference in carcinogenicity.

In this paper the pathological and biochemical changes in rat and mouse liver and kidney, with particular regard to peroxisome proliferation, were determined after inhalation exposure to PER. Blood levels of TCA in rats and mice exposed to PER were also determined. The animal strains and dose levels used in the 1985 PER bioassay (Mennear *et al.*, 1986) were adopted in order to assess the relevance of our results to the development of tumors in these animals.

METHODS

Materials

1,1,2,2-Tetrachloroethylene (Analaar grade, 99.9%) and trichloroacetic acid (Analaar grade, 99% pure) were obtained from BDH Chemicals PLC (Poole, Dorset,

UK). Biochemicals were obtained from Sigma Chemical Co. (Poole, Dorset, UK).

Animals

Male and female Fischer 344 rats (160–180 g) and male and female B6C3F1 mice (23–25 g) were supplied by Charles River (Margate, Kent, UK). Animals were multiply housed in suspended stainless steel wire mesh cages in long-term inhalation exposure chambers, equipped with a 12-hr light cycle, prior to, during, and after exposure. They received food (PCD diet, Special Diets Services Ltd., Witham, Essex, UK) and water *ad libitum* before and after, but not during exposure.

Exposure to PER for up to 28 Days

Exposure. Male and female rats and mice (5 per group) were exposed to concentrations of 200 or 400 ppm of PER for 6 hr/day for 14, 21, or 28 consecutive days. Control animals were exposed to air only, but otherwise were treated in a manner similar to that of the test animals.

Exposures were whole body in stainless steel chambers (Doe and Tinston, 1981) having an internal volume of approximately 3.4 m³. The chambers were air conditioned to have a nominal temperature of 22°C and relative humidity of 40–60%. The air flow through the chambers was 300 liters/min. Atmospheres were generated by passing vaporized PER into the input air of the chambers.

Atmospheres in the test chambers were analyzed for PER by gas chromatography (GC) on a Hewlett-Packard 5880A GC (flame ionization detector) fitted with a Porapak Q5 column (1.8 m × 4 mm). The column temperature was 195°C, helium carrier gas 50 ml/min.

Eighteen hours after the last exposure period, animals were killed by overexposure to halothane (Fluothane, Imperial Chemical Industries PLC, Pharmaceuticals Division) and exsanguinated. The livers and kidneys were rapidly removed, weighed, and then divided to provide tissue for light microscopy, electron microscopy, and biochemical analysis.

Light microscopy. Slices of liver and kidney were fixed in 10% neutral buffered formal saline, dehydrated through an ascending ethanol series, and embedded in paraffin wax. Sections (5 µm) were cut and stained with hematoxylin and eosin.

Electron microscopy. Tissues were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer, dehydrated, and embedded in epoxy resin. Sections (1 µm) were cut and stained with 1% toluidine blue in 1% borax for light microscopy. Areas were selected from the centrilobular regions of the livers and the S3 regions of the proximal tubules of the kidney for electron microscopy. Ultrathin sections of these areas were stained with uranyl acetate

TABLE I

PEROXISOMAL CYANIDE-INSENSITIVE PALMITOYL COENZYME A OXIDATION IN RAT AND MOUSE LIVER AND KIDNEY AFTER EXPOSURE TO PER

		CN-insensitive palmitoyl CoA oxidation (nmol/min/mg protein)			
Concentration (ppm)	Duration (days)	Liver		Kidney	
		Mouse	Rat	Mouse	Rat
Male					
0	14-28	5.16 $\pm$ 1.06*	10.26 $\pm$ 0.51	5.57*	2.37 $\pm$ 0.29
200	28	11.19 $\pm$ 4.46**	12.95 $\pm$ 0.93*	6.92	2.98 $\pm$ 0.21**
400	14	11.98 $\pm$ 2.36**	13.68 $\pm$ 1.68**	7.69	2.97 $\pm$ 0.65
400	21	13.90 $\pm$ 3.27**	12.94 $\pm$ 0.81**	8.70	2.44 $\pm$ 0.49
400	28	18.64 $\pm$ 5.61**	13.61 $\pm$ 0.89**	8.18	2.76 $\pm$ 0.33
Female					
0	14-28	9.01 $\pm$ 1.62	12.62 $\pm$ 0.77	2.48	1.98 $\pm$ 0.21
200	28	16.68 $\pm$ 3.52**	15.76 $\pm$ 1.06**	2.85	3.11 $\pm$ 0.45**
400	14	14.40 $\pm$ 2.27**	14.90 $\pm$ 1.91*	2.59	2.56 $\pm$ 0.28**
400	21	18.74 $\pm$ 1.68**	15.31 $\pm$ 2.31*	2.28	2.68 $\pm$ 0.26**
400	28	17.99 $\pm$ 2.35**	14.14 $\pm$ 1.90	2.49	2.41 $\pm$ 0.19**

Note. Control animals were exposed to air only for 14, 21, or 28 days.

* Values are $\bar{x} \pm$ SD, $n = 5$ except for controls, where $n = 15$.

* Mouse kidneys were pooled according to group; values are $\bar{x}$.

* Statistically significant, $p < 0.05$.

** Statistically significant, $p < 0.01$.

and lead citrate and viewed and photographed in a JOEL JEM 100CX electron microscope. Morphometric analysis of peroxisomes was performed according to the general principles of Weibel *et al.* (1964) on electron micrographs of areas of cytoplasm at a magnification of 25,000.

Biochemical analysis. Sections of liver and kidney remaining after tissue had been taken for light and electron microscopy were placed in ice-cold sucrose (250 mM) EDTA (5.4 mM) Tris-HCl (20 mM) buffer, pH 7.4. Mouse kidneys were pooled according to group. Homogenates (25% w/v approximately) were prepared using a Teflon glass homogenizer at 4°C. Homogenates were centrifuged at 3000g for 5 min at 4°C. The supernatants from the kidney homogenates were stored at -70°C until used. Supernatants from the liver homogenates were further centrifuged at 15,000g for 15 min at 4°C, as described by Elcombe *et al.* (1985). The supernatants were discarded and the pellets (containing peroxisomes) were resuspended in the above buffer and stored at -70°C.

The protein content of the liver and kidney fractions was determined by the method of Lowry *et al.* (1951). The activities of the peroxisomal enzymes catalase and cyanide-insensitive palmitoyl coenzyme A oxidase were determined by the methods of Beers and Sizor (1952) and Brodfman *et al.* (1979), respectively.

TCA Concentrations in Blood after Exposure to PER

Rats and mice were exposed to 400 ppm PER for up to 6 hr. Animals killed at time points of less than 6 hr were exposed in glass desiccators at a flow rate of 5-10 liters/min. Those exposed for the full 6 hr were housed in the long-term chambers described above.

Atmospheres were generated by vaporizing PER into the air stream and were monitored by gas chromatography. Groups of three rats or three mice were killed by exposure to CO₂ and bled by cardiac puncture at intervals from the start of exposure until 48 hr postexposure (see Fig. 2). TCA was extracted from blood as described by Proust *et al.* (1985) and the methylated samples were analyzed on a Hewlett-Packard 5890A gas chromatograph fitted with an electron capture detector. A glass column (2 m x 2 mm), packed with Porapak PS and operated at 180°C with a nitrogen carrier gas flow of 25 ml/min, was used for the analysis. Under these conditions TCA had a retention time of 5.7 min. The limit of detection for TCA in blood was 0.2 µg/mL.

Statistics

Values were tested for statistical significance using the two-tailed Student *t* test.

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RESULTS

Effects of Exposure to PER

The mean analyzed concentrations of PER for the 28 days of exposure were 193 and 389 ppm for the rats and 196 and 395 ppm for the mice. These were close to the target levels of 200 and 400 ppm. No significant clinical abnormalities were seen in rats or mice exposed to either concentration of PER.

Liver

Exposure of B6C3F1 mice to 400 ppm PER for either 14, 21, or 28 days resulted in small but statistically significant increases in liver/body weight ratios up to 1.2- and 1.3-fold in males and females, respectively. F344 rats exposed to PER showed no changes in liver/body weight ratios.

Cyanide (CN)-insensitive palmitoyl CoA oxidase, a marker for peroxisomal β -oxidation was significantly increased in mouse liver after exposure to PER (Table 1). This enzyme increased to a similar level in males and females but the control rate of CN-insensitive palmitoyl CoA oxidation was higher in females. Therefore the increase over control rates was lower in females than males and maximum response (seen after 28 days exposure to 400 ppm) was a 3.6-fold increase in males and a 2.1-fold increase in females.

In contrast, only small increases in CN-insensitive palmitoyl CoA oxidation were observed following treatment of F344 rats with PER (Table 1) although these were sometimes statistically significant. The maximum increase (1.3-fold) was in males exposed to 400 ppm for 28 days. The basal activity of CN-insensitive palmitoyl CoA oxidation was noted to be approximately 2-fold greater in F344 rats than in B6C3F1 mice (Table 1).

Catalase, another peroxisomal enzyme, was largely unaffected in mice and rats ex-

TABLE 2

PEROXISOMAL CATALASE ACTIVITY IN RAT AND MOUSE LIVER AFTER EXPOSURE TO PER

Concentration (ppm)	Duration (days)	Catalase (ksec ⁻¹ mg protein ⁻¹)	
		Mouse	Rat
Male			
0	14-28	1.05 ± 1.17 ^a	1.62 ± 0.26
400	14	1.12 ± 0.19	1.88 ± 0.57
400	21	1.30 ± 0.17 ^a	1.62 ± 0.21
400	28	1.44 ± 0.34 ^a	1.90 ± 0.13
Female			
0	14-28	1.79 ± 0.12	1.56 ± 0.23
400	14	1.56 ± 0.09	1.46 ± 0.15
400	21	1.85 ± 0.05	1.77 ± 0.30
400	28	1.76 ± 0.28	1.59 ± 0.24

Note. Control animals were exposed to air only 14, 21, or 28 days.

* Values are $\bar{x} \pm$ SD, $n = 5$ except for controls, where $n = 15$.

* Statistically significant, $p < 0.05$.

posed to PER (Table 2). The only increases (up to 1.4-fold) were observed in male mice exposed to 400 ppm.

By light microscopy the livers of mice exposed to 400 ppm PER showed centrilobular eosinophilia and centrilobular fatty vacuolation. Both effects were seen to a similar extent in males and females and the numbers of animals affected increased from 14 to 28 days. Similar effects on lipid were seen at the electron microscope level in mice exposed to 200 ppm for 28 days or 400 ppm for 14, 21, or 28 days. Extensive lipid accumulation was observed in centrilobular hepatocytes. The lipid was present in the form of large droplets, 2- to 5- μ m diameter, lying free in the cytoplasm of the cells (macrovesicles), and small droplets, 0.1- to 0.5- μ m diameter, contained within the cisterna of the endoplasmic reticulum (microvesicles). Figure 1 shows the ultrastructural appearance of a centrilobular hepatocyte from (a) an untreated male mouse and (b) a male mouse exposed to 400 ppm PER for 28 days. Electron microscopy showed proliferation of peroxisomes in the

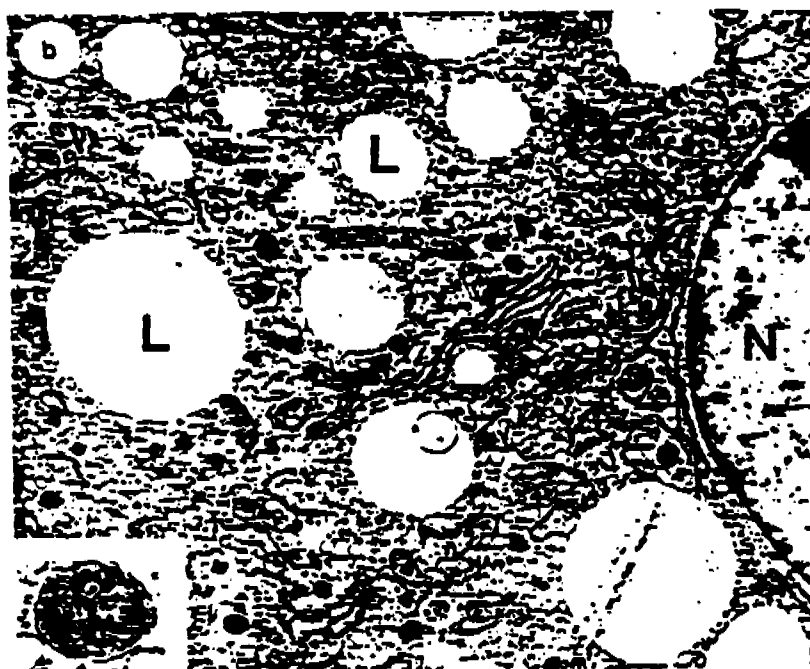


FIG. 1. (a) Ultrastructural appearance of a centrilobular hepatocyte from an untreated male B6C3F1 mouse showing nucleus (N) and peroxisomes (P), $\times 6300$. (b) Ultrastructural appearance of a centrilobular hepatocyte from a male mouse exposed to 400 ppm PER 6 hr/day for 28 days. The cell shows an accumulation of lipid in the form of large droplets (L) and small vesicles and a proliferation of peroxisomes (P). The nucleus is seen at (N), $\times 6300$. Insert: higher magnification of peroxisome showing electron dense core, $\times 19,800$.

TABLE 3
MORPHOMETRIC ANALYSIS OF HEPATIC PEROXISOMES IN MICE AND RATS EXPOSED TO PER

Exposure		Peroxisome volume (% cytoplasm)			
Concentration (ppm)	Duration (days)	Mouse		Rat	
		Male	Female	Male	Female
0	28	2.5 ± 0.6*	2.5 ± 0.3	3.1 ± 0.1	3.3 ± 0.7
200	28	5.2 ± 1.5*	4.4 ± 0.6*	3.7 ± 1.3	4.7 ± 1.9
400	14	4.9 ± 0.7*	4.9 ± 1.5*	2.3 ± 0.6	2.7 ± 0.5
400	21	5.4 ± 1.3*	4.8 ± 0.9*	2.8 ± 0.7	3.2 ± 1.1
400	28	6.0 ± 1.4*	4.8 ± 1.2*	3.4 ± 1.4	3.4 ± 1.0

* Values ($\bar{x} \pm SD$, $n = 5$) are calculated from three micrographs per animal, with 375 points applied to each micrograph.

* Statistically significant, $p < 0.01$.

centrilobular region of the mouse liver (Fig. 1b, Table 3). The proliferated peroxisomes were small ($<0.5 \mu\text{m}$) and the majority retained the central nucleoid (Fig. 1b, insert). Exposure to 400 or 200 ppm resulted in statistically significant increases in the volume of cytoplasm occupied by peroxisomes (Table 3).

Exposure of male mice to 200 or 400 ppm PER also resulted in a decrease in mitochondria after 14 days but this was followed by mitochondrial proliferation in those animals subsequently exposed to 400 ppm. The effect was not seen in females. Concomitant with these changes, exposure at either level for any of the time periods investigated resulted in a decrease in the amount of normal rough endoplasmic reticulum in the cells.

Light microscopic examination of livers from rats exposed to PER showed centrilobular hypertrophy in both sexes with a concomitant loss of glycogen. The effects in males were of similar intensity in both the 200- and 400-ppm dose groups and there was little evidence of progression of the lesion from 14 to 28 days in the 400-ppm group. Results suggest that the males were more sensitive to the liver hypertrophic effects of PER since no effect was seen in females exposed to 200 ppm for 28 days.

Electron microscopy showed a time-dependent proliferation of smooth endoplasmic reticulum in the liver in both sexes which correlated well with the centrilobular hypertrophy. The males were more susceptible than the females. There was a dose- or time-dependent increase in peroxisomes in the livers of either sex (Table 3).

Kidney

No increases in kidney/body weight ratios were seen in rats and mice exposed to PER.

The effect of PER on peroxisomal cyanide-insensitive palmitoyl CoA oxidation in rats and mice is shown in Table 1. Insufficient mouse kidney tissue precluded the measurement of this marker in individual animals. Consequently values could not be tested for statistical significance. Slight increases were seen in β -oxidation in male mouse kidney, the maximum being a 1.6-fold increase after 21 days exposure to 400 ppm. Small increases in this marker were also observed in female rat kidneys after exposure to PER (Table 1) up to a maximum of 1.6-fold.

There was no effect of PER on renal catalase activity in rats or mice of either sex (data not shown).

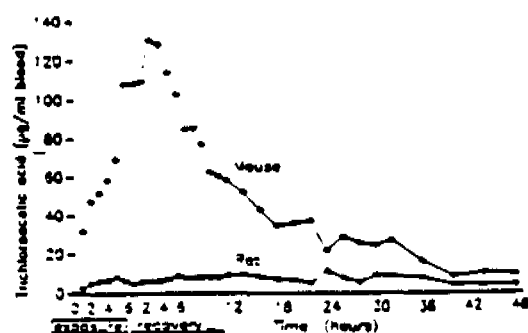


FIG. 2. Blood levels of TCA in mice and rats exposed to PER (400 ppm) for 6 hr and recovery. Values are means with three animals to each time point.

No compound-related changes were observed in the kidneys of either species at the light or electron microscope level.

Blood Levels of TCA after Exposure to PER

Blood levels of TCA in rats and mice during and after a 6-hr exposure to PER (400 ppm) are shown in Fig. 2. Peak blood levels of TCA (approximately 130 $\mu\text{g/ml}$) in mice were reached 3–4 hr after the end of the exposure period and thereafter declined with a half-life of 7–8 hr. Forty hours after exposure, levels persisted at 8–10 $\mu\text{g/ml}$. In contrast blood levels in the rat reached a plateau of approximately 7 $\mu\text{g/ml}$ after 3 hr of exposure declining to 4 $\mu\text{g/ml}$ 48 hr after the end of exposure. Comparison of the concentrations of TCA to which the two species were exposed, by calculation of the area under the curves, shows the mouse to have been exposed to 6.7-fold more TCA than the rat.

DISCUSSION

The hepatocarcinogenicity of PER in the mouse has been known for some years but no satisfactory mechanism, either genotoxic or epigenetic, has so far been proposed. The first step in the metabolism of PER is believed to be oxidation to an epoxide (Reichert, 1983)

and the metabolites which have been identified support this assumption (Yllner, 1961; Daniel, 1963; Bonse *et al.*, 1975; Sakamoto, 1976). Alkylation of nucleotides by reactive epoxides has been described for other chlorinated alkenes such as vinyl chloride (Laib and Bolt, 1977) and vinylidene chloride (Reitz *et al.*, 1980). DNA binding has not however been demonstrated after treatment of rats and mice with PER (Schumann *et al.*, 1980) nor does PER induce gene mutations in bacteria (Bartsch *et al.*, 1979; Greim *et al.*, 1975; Bronzetti *et al.*, 1983). These observations led to proposals (Schumann *et al.*, 1980) that PER-induced liver tumors in B6C3F1 mice are a result of recurrent cytotoxicity and enhancement of the high spontaneous incidence of liver tumors found in this strain of mouse.

The results of the present study suggest an alternative hypothesis for the mechanism of PER-induced carcinogenicity, that of peroxisome proliferation leading to cancer formation via an epigenetic mechanism (Reddy *et al.*, 1980). Exposure of male and female mice to PER for up to 28 days resulted in a significant proliferation of peroxisomes in the liver as measured by peroxisomal β -oxidation (Table 1) and morphometric analysis of electron micrographs (Table 3). The induction of peroxisomal β -oxidation in this study was not accompanied by increases in catalase (Table 2). This phenomenon has been observed after administration of other peroxisome proliferators (Cohen and Grasso, 1981; Reddy and Lalwani, 1983, for review) and is believed to lead to increased levels of hydrogen peroxide in the cell, causing oxidative damage, cytotoxicity, and possibly DNA damage. However, a definite link between such changes and the eventual development of cancer remains to be established.

Although increases in the activity of peroxisomal enzymes were observed in the livers of rats after treatment with PER (Table 1), these were slight compared to the changes seen in the mouse and could not be corroborated by electron microscopy (Table 3). Sim-

ilar results after treatment of rats and mice with PER have recently been reported by Goldsworthy and Popp (1987). A sixfold increase in hepatic CN-insensitive palmitoyl CoA oxidase was observed in B6C3F1 mice dosed PER by gavage at 1000 mg/kg for 10 days. No such increase was seen in F344 rats. This species difference in peroxisome proliferation is identical to that found by Elcombe *et al.* (1985) after TRI was dosed by gavage to mice and rats. There were however differences in the pathology of treated livers between the present study and that reported by Elcombe *et al.* (1985) for TRI. PER-induced peroxisome proliferation was observed in the centrilobular region of mouse liver. The induced peroxisomes were small and retained the nucleoid core, whereas after TRI treatment they generally lacked the nucleoid core. Exposure to PER also resulted in a concomitant accumulation of lipid in centrilobular cells with periportal cells unaffected. The reasons for these differences are unknown but may be due to the effects of other metabolites or the parent chemical.

TCA, the major metabolite of PER (De-kant *et al.*, 1985), is a known hepatic peroxisome proliferator in both rats and mice (Elcombe, 1985) and is the metabolite responsible for increased peroxisomes in mice exposed to TRI in previous studies (Elcombe *et al.*, 1985). In the present study, TCA arising from metabolism of PER only induced hepatic peroxisome proliferation in mice because of the much higher concentrations of this metabolite in mouse blood. The lack of a response in rats indicates that a threshold concentration of TCA has to be reached in order to induce peroxisome proliferation in rodent liver. The low blood levels of TCA observed in rats compared with mice correlates with the lower rate of oxidative metabolism of PER in rats than mice (Schumann *et al.*, 1980; Ikeda and Ohtsui, 1972).

Recent studies have confirmed that TCA is in fact a carcinogen in B6C3F1 mice (Herren-Freund *et al.*, 1986). TCA dosed to male mice in drinking water at 5 g/liter for 61 weeks pro-

duced a 50% tumor incidence compared to a 5% incidence in the control group. Peroxisome proliferation was observed in the livers of treated animals. Thus the species difference in the carcinogenicity of PER between rats and mice may be explained by the marked difference in blood levels of TCA and a mechanism which induces peroxisome proliferation.

The effect of PER on the kidney in mice and rats was minimal (Table 1). No compound-related changes were seen at the light or electron microscope level in regions of the nephron where peroxisomes are known to be most prevalent (Beard and Novikoff, 1969). Similarly the increases observed in peroxisomal enzymes were slight and not related to dose or exposure. Peroxisome proliferation is therefore unlikely to play a role in the carcinogenicity of PER in the rat kidney and further investigations are needed to establish an alternative mechanism.

Metabolism of PER in man is known to occur at a very slow rate (Fernandez *et al.*, 1976; Monster *et al.*, 1979). It is also a saturable process, saturation occurring at the low inhalational exposure level of 100 ppm (Ikeda *et al.*, 1972; Ohtsui *et al.*, 1983). Consequently man is exposed to lower concentrations of TCA than mice or rats. Furthermore, TCA does not induce peroxisome proliferation *in vitro* in human hepatocytes (Elcombe, 1985); indeed the response of primates to the induction of peroxisome proliferation by other agents is generally much lower than that of rodent species (Cohen and Grasso, 1981; Reddy and Lalwani, 1983).

In conclusion this study demonstrates that quantitative differences in the metabolism of PER to TCA in mice and rats lead to proliferation of peroxisomes in the livers of mice but not rats. The known carcinogenicity of TCA in B6C3F1 mice and the correlation between hepatic peroxisome proliferation and cancer in rodents strongly suggests that TCA-induced peroxisome proliferation is the basis of the species difference in hepatocarcinogenicity of PER. The limited capacity of humans

to metabolize PER coupled with an intrinsic deficiency in response to TCA as a peroxisome proliferator indicates that PER is unlikely to cause hepatocellular carcinoma in man.

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REFERENCES

- BARTSCH, H., MALAVEILLE, C., BARBIN, A., AND PLANCHE, G. (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadiene and dichlorobutenes produced by rodent or human liver tissues. Evidence for oxirane formation by P450 linked microsomal mono-oxygenases. *Arch. Toxicol.* 41, 249-277.
- BEARD, M. E., AND NOVIKOFF, A. B. (1969). Distribution of peroxisomes (microbodies in the nephros of the rat. A cytochemical study. *J. Cell Biol.* 42, 501-518.
- BEERS, R. F., AND SIZER, J. W. (1952). A spectrophotometric method of measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* 195, 133-140.
- BONSE, G., URBAN, TH., REICHERT, D., AND HENSCHLER, D. (1975). Chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. *Biochem. Pharmacol.* 24, 1829-1834.
- BRONFMAN, M., INESTROSA, N. C., AND LEIGHTON, F. (1979). Fatty acid oxidation by human liver peroxisomes. *Biochem. Biophys. Res. Commun.* 88, 1030-1036.
- BRONZETTI, G., BAUER, C., CORSI, C., DEL CARRATORE, R., GALLI, A., NIERI, R., AND PAOLINI, M. (1983). Genetic and biochemical studies on perchloroethylene "in vitro" and "in vivo." *Mutat. Res.* 116, 323-331.
- COHEN, A. J., AND GRASSO, P. (1981). Review of the hepatic response to hypolipidaemic drugs in rodents and assessment of its toxicological significance to man. *Food Cosmet. Toxicol.* 19, 585-605.
- DANIEL, J. W. (1963). The metabolism of ^{35}Cl -labeled trichloroethylene and tetrachloroethylene in the rat. *Biochem. Pharmacol.* 12, 795-802.
- DEKANT, W., HANG, R., AND HENSCHLER, D. (1985). Absorption, elimination and metabolism of tetrachloroethylene. *Vaunyn-Schmiedeberg's Arch. Pharmacol. (Suppl.)* 329, R24.
- DOE, J. E., AND TINSTON, D. J. (1981). Novel chambers for long term inhalation studies. In *Proceedings of the Inhalation Toxicology and Technology Symposium* (B. K. J. Leong, Ed.), pp. 77-88. Ann Arbor Science Pub., Ann Arbor, MI.
- ELCOMBE, C. R. (1985). Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: A biochemical human hazard assessment. *Arch. Toxicol. (Suppl.)* 8, 6-17.
- ELCOMBE, C. R., ROSE, M. S., AND PRATT, I. S. (1985). Biochemical, histological, and ultrastructural changes in rat and mouse liver following the administration of trichloroethylene: Possible relevance to species differences in hepatocarcinogenicity. *Toxicol. Appl. Pharmacol.* 79, 365-376.
- FERNANDEZ, J., GUBERMAN, E., AND CAPEROS, J. (1976). Experimental human exposures to tetrachloroethylene vapour and elimination in breath after inhalation. *Amer. Ind. Hyg. Assoc. J.* 37, 143-150.
- GOLDSWORTHY, T. L., AND POPP, J. A. (1987). Chlorinated hydrocarbon induced peroxisome enzyme activity in relation to species and organ carcinogenicity. *Toxicol. Appl. Pharmacol.* 88, 225-233.
- GREEN, T., AND PROUT, M. S. (1985). Species differences in response to trichloroethylene. II. Biotransformation in rats and mice. *Toxicol. Appl. Pharmacol.* 79, 401-411.
- GREIM, M., BONSE, G., RADWAN, Z., REICHERT, D., AND HENSCHLER, D. (1975). Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. *Biochem. Pharmacol.* 24, 2013-2017.
- HERREN-FREUND, S. L., PEREIRA, M. A., OLSEN, G., AND DE ANGELO, A. B. (1986). The carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid in mouse liver. *Proc. Amer. Assoc. Cancer Res.* 27, 91.
- IKEDA, M., AND OHTSUKI, H. (1972). A comparative study of the excretion of Fujiwara reaction-positive substances in urine of humans and rodents given trichloro or tetrachloro-derivatives of ethane and ethylene. *Br. J. Ind. Med.* 29, 99-104.
- IKEDA, M., OHTSUKI, H., IMAMURA, T., AND KOMOIKE, Y. (1972). Urinary excretion of total trichloro-compounds, trichloroethanol and trichloroacetic acid as a measure of exposure to trichloroethylene and tetrachloroethylene. *Br. J. Ind. Med.* 29, 328-333.
- LAIB, R. J., AND BOLT, H. M. (1977). Alkylation of RNA by vinyl chloride metabolites *in vitro* and *in vivo*: Formation of 1'-N-etheno-adenosine. *Toxicology* 8, 185-195.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
- MENNEAR, J. H., CLARKE, W., RAGAN, H., AND MILLER, R. (1986). Toxicology and carcinogenicity

- studies of inhaled tetrachloroethylene (PERC) in male and female F344 rats and B6C3F₁ mice. *Toxicologist* 6, 135.
- MONSTER, A. C., BOERSMA, G., AND STEENWEG, H. (1979). Kinetics of tetrachloroethylene in volunteers: Influence of exposure concentration and work load. *Int. Arch. Occup. Environ. Health* 42, 303-309.
- National Cancer Institute (1976). *Carcinogenesis Bioassay of Trichloroethylene*. CAS No. 79-01-6. DHEW pub. No. (NIH) 76-802.
- National Cancer Institute (1977). *Bioassay of Tetrachloroethylene for Possible Carcinogenicity*. DHEW pub. No. 77-813.
- National Toxicology Program (1983). National Toxicology Program draft report abstracts on nine chemical carcinogenesis animal bioassays. *Chem. Regul. Rep.* 6, 767-768.
- OHITSUKI, T., SATO, K., KOIZUMI, A., KUMAI, M., AND IKEDA, M. (1983). Limited capacity of humans to metabolize tetrachloroethylene. *Int. Arch. Environ. Health* 51, 381-390.
- PROUT, M. S., PROVAN, W. M., AND GREEN, T. (1985). Species differences in response to trichloroethylene. I. Pharmacokinetics in rats and mice. *Toxicol. Appl. Pharmacol.* 79, 389-400.
- REDDY, J. K., AZARNOFF, D. L., AND HIGHTTE, C. E. (1980). Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature (London)* 283, 397-398.
- REDDY, J. K., AND LALWANI, N. D. (1983). Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidaemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* 12, 1-58.
- REDDY, J. K., KRISHNAKANTHA, T. P., AND TAO, M. S. (1975). Microbody (peroxisome) proliferation in mouse kidney induced by methyl clofenapate. *Virchows Arch. B Cell Pathol.* 17, 295-306.
- REDDY, J. K., WARREN, J. R., REDDY, M. K., AND LALWANI, N. D. (1982). Hepatic and renal effect of peroxisome proliferators: Biological implications. *Ann. N.Y. Acad. Sci.* 386, 81-110.
- REICHERT, D. (1983). Biological actions and interactions of tetrachloroethylene. *Mutat. Res.* 123, 411-429.
- REITZ, R. H., WATANABE, P. G., MCKENNA, M. J., QUAST, J. F., AND GEHRING, P. J. (1980). Effects of vinylidene chloride on DNA synthesis and DNA repair in the rat and mouse: A comparative study with dimethylnitrosamine. *Toxicol. Appl. Pharmacol.* 52, 357-370.
- SAKAMOTO, N. (1976). Metabolism of tetrachloroethylene in guinea pigs. *Japan. J. Health* 18, 11-16.
- SCHUMANN, A. M., QUAST, J. F., AND WATANABE, P. G. (1980). The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity. *Toxicol. Appl. Pharmacol.* 55, 207-219.
- WEIBEL, E. R., STAUBLI, W., GNAGE, H. R., AND HESS, F. (1964). Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model stereological methods and normal morphometric data for rat liver. *J. Cell Biol.* 21, 68-91.
- YLLNER, S. (1961). Urinary metabolites of 14C-tetrachloroethylene in mice. *Nature (London)* 191, 820.

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The Cytotoxicity of Trichloroethylene (TCE) and Its Metabolites, Trichloroacetic Acid (TCA) and Dichloroacetic Acid (DCA), in Mouse Liver. ¹G.L. Sargent-Francis, ¹M.A. Perumal, ²G. Glenn, and ¹A.S. DeAngelis. ¹U.S. EPA, Cincinnati, OH 45226 and ²Pathology Associates, Inc., Cincinnati, OH 45229 (Introduced by T.V. Lundy)

TCE, which is used as an industrial solvent, has been shown to be carcinogenic in mouse liver. The metabolism of TCE results, in part, in the formation of chlorinated acetic acids such as TCA and DCA. These chlorinated acetic acids have been shown to be mutagenic and to induce peritoneal proliferation. Therefore, we determined the ability of TCE, TCA and DCA to act as tumor promoters in mouse liver. Male B6C3F₁ mice were administered i.p. 2.5 ug/g subcutaneous-urea (SNU) on day 13 of age. At 28 days of age, the mice were placed on drinking water containing TCE (3 and 30 ug/l), TCA (2 and 3 g/l), or DCA (2 and 3 g/l). All drinking water had a final pH of 7.3. The animals were killed after approximately 61 weeks of exposure (63 weeks of age). Both DCA and TCA at a dose of 3 g/l were carcinogenic without prior initiation with SNU and resulted in 84% and 56%, respectively, of the animals with tumors. Exposure to TCE at 30 ug/l without prior SNU initiation resulted in 19% of the animals with tumors. Pre-treatment with SNU did not increase the tumor incidence in the TCE, TCA or DCA treated mice. SNU treatment by itself induced tumors in 11% of the animals. Only 1% of the untreated animals had tumors. All three of these chemicals induced peritoneal proliferation in mouse liver with TCA being the strongest inducer followed by DCA and then TCE. Thus the carcinogenic pathway of these chemicals appears not to parallel their relative ability to induce peritoneum. This document does not necessarily reflect EPA policy.

PHASE II TRIAL OF 3-METHYLPOLYMERIZING (MUP)
HEAD AND NECK CANCER. U.C. Vogel, M.D. 70-
east, L.B. Jernale, G. Zahner. The University
and Ann Arbor VA Medical Centers. Ann Arbor
MUP is a polar compound which demonstrates
activity and cell differentiation in vitro.
In Phase I certain dose-limiting toxicity
observed primarily with weekly doses once
between and vomiting and cardiac toxicity at
higher doses. In this trial, the initial
1000 mg/M² IV biweekly, days 1-5 (3 pts) and
pts. In the absence of toxicity doses was
mg/M²/cycle (max. 1000 mg/M²). Dose was re-
covered (gr 3) nausea and vomiting. The end
(range 44-49) and performance status 00
pts had no prior therapy, b-radiation therapy (X
ray (G1), 3-surgery and RT, b-surgery - RT
had advanced bone marrow reserve, serum or
normal SGOT, SGPT, serum bilirubin, albumin
(A1). Pts with heart disease were not elig-
ible pts (2 too early) 11 had progression
treated with 500 mg/M² was withdrawn from
severe hepatotoxicity. Nausea and vomiting
pts and was severe in 4. Hepatotoxicity a
liver function test (LFT) elevated 3 X has
9/20 cycles; elevated SGOT in 9/20 cycles.
AF-3/20 cycles. One patient was removed for
elevation >100 X baseline. Two pts had pa-
rameterization which delayed recurrence. 3
could said LFT abnormalities 4 months after
pains occurred in 2 pts (WBC 3,400 and 3.2
thrombocytopenia in 1 (platelets 42,000 cu
mm). No cardiotoxicity was observed. The
case that MUP as used in this trial was
in the treatment of squamous cell and
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CELL CELL-RECEPTOR INITIATION OF HEPATOCARCINOGENESIS BY
(+)BENZO(a)PYRENE DIB-EPoxide I (BPDE). RJ Reddy, M.
Kandam, M Kandam, Department of Pathology and
Lieschinger Cancer Research Center, University of South.
Carolina, Choral Hill, SC 27314

The sensitivity of proliferating cells to carcinogenesis may be influenced by the phase of the cell cycle in which carcinogen-induced damage is incurred. The ability of cells to repair DNA damage, and replication of damaged DNA. To investigate the interrelationships of these factors during the initiation of hepatocarcinogenesis, hepatocyte proliferation was induced in F344 rats by a two-thirds partial hepatectomy (PH) and rats received one dose of IPFX (0.02 mg/kg) via the portal vein when hepatocytes were in early G₁ (4h) or early S (15h). Carcinogenesis was promoted with dietary phenobarbital and incidence was measured 45 weeks post-treatment. Repair of DNA damage and the effect of IPFX on DNA synthesis were also evaluated by utilizing an ELISA for UVA-GMA adducts and by measuring the specific activity of ³-thymidine incorporation into DNA. Rats treated when hepatocytes were in early S had a significantly higher incidence of hepatocytic neoplasms (80%) than did rats treated in early G₁ (22%). The peak of DNA synthesis after PH was delayed 24 h in livers treated when hepatocytes were in early G₁. In the same livers, adducts were reduced by 50% at the time of this peak of DNA synthesis. These preliminary results suggest that hepatocyte susceptibility to initiation of carcinogenesis by IPFX is cell cycle-dependent, with hepatocytes in early S being more sensitive than hepatocytes in early G₁. For G₁ phase hepatocytes the delay in DNA synthesis following IPFX treatment may have reduced the risk by providing additional time for the repair of damaged DNA. Supported by S-1 Grant CA33238 and CA33717.

**SPECTRA OF 2-ACTIVE-2-ACETYLANTHROPOLYMER
MUTATIONS IN THE MAMMALIAN NERF LOCUS. A.
L.N. Stiglmayr, G. Ulrich, L. Chou, &
Grunberger, California University, Institute
Research and Department of Biological Sci-
ence 10012.**

We are examining the spectrum of ~~the~~ by the carcinogen, 1-methyl-3-acetylamin- at the dihydrofolate reductase locus of C. every cell. We have obtained 29 isolates starting with a SHR⁺ hamster cell line the locus is detected by Southern blot anal- and parental SHAs digested with SalII a mixed probe of 18 genomic and cDNA frag- mutants showed a banding pattern identical strain and are likely to carry point muta- point mutations were localized by restriction lyzymes; in one, a SalI site is gone; a SalI site is gone; were lost. In a third transversion creating a new SHRII site is gone. Furthermore, six deletion mutants. In the other deletion mutants, however, the ends of the gene are no longer collinear: translocation, inversion, or duplication. by RFLP the ratio of deacetylated (88%) to (12%) of mutants found in the parental is- vant with AAf. The ratio of point muta- gross structural changes (28%) is similar of-AAf and C-AAf mutants forms identical correlation between the type of conforma- cused by the mutagen and the structural n- work was supported by NIH Grant CA-17328

3/15 Jim ES
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HSIA

HALOGENATED SOLVENTS INDUSTRY ALLIANCE

1225 19th Street, N.W., Suite 300, Washington, D.C. 20036-2411 • (202) 223-5590

RECEIVED

March 13, 1990

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Stationary Source
Division
Air Resources Board

Mr. Donald J. Ames
Assistant Chief
Stationary Source Division
Air Resources Board
Attn: Perchloroethylene
P. O. Box 2815
Sacramento, CA 95812

Dear Mr. Ames:

On February 1, 1990, HSIA submitted comments on the draft technical support document for the proposed identification of perchloroethylene as a toxic air contaminant. On page 26 of these comments there is a typographical error. The first sentence on page 26 currently reads "[t]his calculation shows, in other words, a potential risk of 98,000 in 1,000,000." The correct sentence should read "[t]his calculation shows, in other words, a potential risk of 980,000 in 1,000,000."

Enclosed is a corrected page 26. Please insert this corrected page into the HSIA comments.

Sincerely,



Thomas A. Cortina
Director of Administration

Enclosure

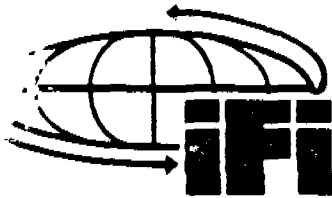
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CORRECTED PAGE

This calculation shows, in other words, a potential risk of 980,000 in 1,000,000. Needless to say, this is a very large and very detectable potential risk. The example of a 30-year exposure at the TLV may be unrealistic, however. Thus, the table presented at the end of our comments shows similar calculations for different fractions of the TLV and for the lower and upper bound of the risk estimates in the draft report.

It can be seen from this table that even the lower bound on the risk estimate range shows extreme (e.g., 7,000 in 1,000,000) potential lifetime risks. Assuming that the exposure scenarios are realistic, either the calculated potential risk is real, does exist, and has gone undetected, or the calculated unit risk estimates are wrong. Past manufacturing plant experience would indicate that particularly the low end of the ppm-year scenarios are very consistent with past practices and may even be under-estimates. For example, drycleaning workers, who have a geometric mean exposure of 22 ppm with a skewed distribution, may have an average exposure generally exceeding 25 ppm. Yet the drycleaning industry has been the subject of numerous epidemiology studies, none of which have detected a risk of the magnitude shown in the table. This is true as well for other industries that use perchlorethylene. Thus, we are left with the conclusion that the unit risks estimates in the draft report vastly overestimate the potential risk to humans.



International Fabricare Institute

12251 TECH ROAD • SILVER SPRING, MD 20904 • (301) 622-1900

January 31, 1990

Mr. Donald J. Ames, Assistant Chief
Stationary Source Division
Air Resources Board
P.O. Box 2815
Sacramento, CA 95812

Stationary Source
Division
Air Resources Board

Dear Mr. Ames:

RE: Comments of the International Fabricare Institute on the Preliminary Draft of the Technical Support Document for "Proposed Identification of Perchloroethylene as a Toxic Air Contaminant"

The International Fabricare Institute and the California Fabricare Institute--respectively the national/international association and the state association for retail drycleaning and laundry businesses--wish to submit the following comments on the Air Resource Board's proposal to identify perchloroethylene as a toxic air contaminant.

At the onset, we wish to express the need for possible additional comments on the future "final draft" report beyond the limits of only "...comments on the Executive Summary and revisions made to preliminary draft report" as expressed in the ARB cover letter. With an official release date of December 28, 1989--and actual receipt at our offices on January 8, 1990--a scant three weeks has been available for review and preparation of comments on a complex and comprehensive document. Given the likely impact of designating perchloroethylene a carcinogenic air pollutant, versus the extremely limited time constraints given for initial comments, we request the opportunity to provide further substantive comment as needed.

I. Impact on the Drycleaning Industry of Designating PCE a Carcinogenic Air Pollutant

According to a national survey of equipment and plant operations conducted in 1988 by IFI, 79.0% of all retail drycleaning plants in the United States use PCE exclusively, while an additional 9% of plants use PCE in addition to another solvent. While no break-out figures are available specifically for California, we would expect that PCE usage would be the same or slightly higher than national figures.

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THE ASSOCIATION OF PROFESSIONAL DRYCLEANERS AND LAUNDERERS

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There are no known or anticipated substitutes for PCE in the drycleaning industry. Of the other solvents currently used, mineral spirits (eg, Stoddard solvent) have been in use since the 1920's, but because of combustibility are prohibited from new installations in virtually all urban and suburban areas of the country.

The limited use of fluorocarbon 113 in drycleaning (approximately 6% of plants) is expected to phase out in the near future because of decreasing availability and rising costs connected with implementation of the Montreal Protocol provisions. Obviously, F-113 is not a viable substitute for PCE.

Similarly, methyl chloroform (1,1,1--trichloroethane)--which after its introduction as a specialized drycleaning solvent two years ago is now used by only approximately 50 plants in the nation--is not a viable substitute because of ozone depletion concerns and its likely inclusion under the Montreal Protocol.

Finally, "emerging" solvents such as HCFC 131a or 141b have chemical and/or physical properties which make them either unusable or potentially unsafe (eg, explosibility) for the drycleaning of textile goods. Additionally, recent statements indicate that HCFCs are being viewed by producers as an interim solution and may be phased out within approximately ten years.

With no viable substitutes available or anticipated for PCE in drycleaning, the central issue then becomes that of potential emission limitations which would be likely to be imposed following designation of PCE as a carcinogenic air pollutant.

One example of possible emission limitations is the South Coast Air Quality Management District's "Screening Risk Calculation" in proposed Rule 223 of March 1989. Using the ARB Technical Support Document (TSD) upper-bound risk estimates for PCE, the SCAQMD's Screening Risk Calculation can be rearranged to project maximum "allowable" daily emissions; these would range from 0.35--1.5 lb/day.

For a typical small drycleaning plant cleaning 1,500 lbs. of garments a week (300 lbs/day), this would equate to an emission factor range of 0.1--0.5 lb PCE/100 lbs. cleaned, or the equivalent of 140,000--700,000 lbs cleaning/drum of PCE.

Emission limits in this range--and their equivalent solvent mileages--far exceed that possible under RACT, BACT, and MACT--in fact, outstrip that of the best operating plant in the nation by an order of magnitude or better.

Our industry has consistently taken the position that if PCE is shown to be a probable human carcinogenic, then appropriate measures should be adopted to protect industry employees and the public. However, we also believe strongly that any such determination must be based on sound scientific data and consistent and rigorous interpretation of any such results.

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Significant evidence has arisen indicating that PCE, while carcinogenic in mice, is unlikely to be a human carcinogen. In concert with this, EPA's Science Advisory Board has suggested that PCE is a specific example of a compound that does not cleanly fit into EPA's current carcinogen ranking system. That ranking system is, of course, now undergoing review with respect to the possible inclusion of additional categories.

The significant questions about the weight of evidence for possible human carcinogenicity of PCE clearly indicate that designation as a carcinogenic air pollutant is unwarranted. Moreover, the incalculable damage to the drycleaning industry which would occur from such a designation could never be recovered.

As an industry, we believe that significant steps can be taken in reducing emissions of PCE in all media...but that designation of PCE as a carcinogenic air pollutant is not only not necessary or warranted, but disastrous.

The impact on drycleaning of a carcinogen designation for PCE is not overstated--nor is our commitment to achieving significant reductions in PCE emissions by working with the Air Resources Board in the development of standards.

II. Comments on ARB Technical Support Document Part A

Rather than the 4,200 drycleaning facilities in California estimated in the TSD, the just-released 1987 U.S. Census of Service Industries reports 2,450 retail drycleaning stores, 916 coin-op laundry/cleaning stores, and 157 industrial laundry facilities in California.

Based on IFI national industry statistics, PCE is used in approximately 88% of retail stores, 15-20% of coin-ops and 2-5% of industrial plants. Applying these distributions gives adjusted figures for PCE drycleaning stores in California of approximately 2,200 retail, 140-200 coin-op, and 3-8 industrial--or approximately 2,300--2,400 facilities overall, a significant reduction from the estimated 4,200 facilities.

The 1987 Census of Service Industries statistics also provide a method for calculating the approximate total PCE consumption by the drycleaning industry. As reported, total retail drycleaning receipts in California for 1987 were approximately \$518 million.

Using an IFI receipt factor for 1987 of \$2.52/lb cleaned--as developed in the annual IFI operating cost survey--gives approximately 205.5 million pounds cleaned per year. This annual poundage figure, multiplied by the 88% of the stores which use PCE, gives a value of approximately 181 million pounds cleaned in retail PCE stores in California.

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Even assuming a relatively low average "solvent mileage" of 8,000 lbs clothes cleaned/700 lb drum of PCE (as opposed to current best estimates of 10,000 lbs/drum), calculated PCE consumption by retail stores would be approximately 7,900 tons per year.

In the absence of current data for PCE consumption factors for coin-op and industrial facilities, using the same ratios between industry sectors as reported by EPA (TSD Part A, Appendix A-2) gives a total for all sectors of drycleaning of approximately 11,000 tons PCE/year, rather than the 18,100 tons/year estimated.

It should be noted that the results of this calculation are consistent by ratio with the reduced number of plants reported in the Census. Similarly, using this method of calculation with a higher number of plants--eg, those estimated in the TSD--would give a higher PCE consumption which would be very close to that originally estimated.

One other likely cause for the higher ARB number for drycleaning PCE use is the data cited by producers of 14,930 tons of PCE "shipped in 1985 from U.S. producers to California for drycleaning." In general, we have found that drycleaning consumption of PCE has frequently been overestimated in the past by PCE producers via the use of numbers such as this.

Specifically, it should be noted that PCE is not shipped directly to drycleaning stores. Instead, it is purchased from solvent distributors from whom the same drycleaning grade PCE is frequently purchased by other users of PCE--ie, businesses outside the drycleaning industry--as the purity level is greater than that of industrial-grade PCE. Unfortunately, the solvent producers will often equate sales of drycleaning-grade PCE to distributors directly with sales to drycleaning plants, when such is not the case. We would be happy to refine these calculations in concert with the ARB as needed.

The estimate in the TSD of air emissions as a fraction of total PCE consumption in drycleaning (ie, 0.88 lb PCE emitted/lb PCE used) is significantly high, most likely because of an under estimation of the quantity contained in hazardous wastes.

Absent a small quantity generator waste exemption in California, these PCE-containing drycleaning wastes are drummed and sent to solvent recycling facilities, such as those of Safety Kleen. As most of the retail drycleaning sector relies on standard-sized cartridge filters as part of their drycleaning system, and distills solvent at fairly constant rates relative to poundage cleaned, the total residual PCE in cartridge and still wastes is relatively constant at approximately 3.2 lbs PCE/100 lbs cleaned.

Solvent mileages of 8,000--10,000--15,000--20,000 lbs cleaned/700 lb drum of PCE are equivalent, respectively, to consumption rates of 8.8--7.0--4.7--3.5 lbs PCE/100 lbs cleaned.

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After subtracting the relatively constant 3.2 lb PCE/100 lbs cleaned waste loss from the above numbers, air emissions equal, respectively, 64%--54%--32%--9% of total PCE use, not the 88% estimated in the document. Again, we would be happy to provide a more in-depth analysis of these factors with ARB staff.

Part A also touches upon indoor air concentrations of PCE, some portion of which may be attributable to residuals in drycleaned articles. As part of this discussion, it is noted that data is available on indoor air levels of PCE from several European studies, but that this data may not be representative of California.

We believe that the ARB is correct in feeling that this data may be non-representative. The common European practice of installing so-called "unit drycleaning shops" in blocks of residential apartment rows has presented problems. Typically, a unit shop is often found with apartments on either side of the plant, above it, and behind it...with no means of ventilation other than through the front door. Fortunately, this is not a common practice in the U.S.

The drycleaning industry is concerned about the possibility of PCE in drycleaned garments contributing to indoor air levels. To this end, we are currently finalizing a draft test program with the cooperation of U.S. EPA. Testing under this program will evaluate possible contributions of drycleaned garments to indoor air levels of PCE and the possible operational changes which might be used to minimize any PCE residuals.

III. Comments on ARB Technical Support Document Part B

The evidence suggestive of PCE's carcinogenicity, when scrutinized carefully, consists primarily of two findings of liver tumors in the B6C3F1 mouse. The scientific community has repeatedly cautioned against reliance on liver tumors in this strain of mice as an indicator of possible carcinogenicity in humans. As one example, researchers have identified a cellular oncogene in this strain, and this has been postulated as the likely cause for its extreme susceptibility to tumors.

Given the absence of any valid finding of carcinogenicity in other animal species--as discussed in the following--let alone in humans, PCE should not be classified as a "potential" (ie, probable) human carcinogen by the Air Resources Board.

Classification of PCE as a probable human carcinogen reverses at least three contrary determinations by EPA and/or its scientific advisors. The U.S. EPA's July 1985 Health Assessment Document (HAD) on PCE concluded:

According to the Agency's Proposed Guidelines for Carcinogen Risk Assessment (published November 1984), the cancer evidence of PCE in animal test systems is limited and the cancer evidence in epidemiologic studies is inconclusive. The overall weight-of-evidence classification for PCE would be Group C, ie., a possible human carcinogen.

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HAD at 1-4, 9-73 (emphasis added). The Agency's Science Advisory Board also concluded in 1984 that "insufficient evidence exists to classify the chemical as a probable carcinogen for humans." Letter from Herschel E. Griffin, M.D. and Norton Nelson, Ph.D. to EPA Administrator Ruckelshaus, January 4, 1985. In addition, EPA's Risk Assessment Forum Classified PCE in Group C. See 50 Federal Register 46887. All of these determinations were made after publication of EPA's 1984 proposed guidelines for classifying carcinogens and reflected the criteria specified in the guidelines.

The critical issue presented by EPA's 1986 HAD Addendum and by the ARB Technical Support Document is whether subsequent experimental findings warrant a reversal of the earlier classification. The Addendum cites two studies in support of its conclusion: (1) the 1977 NCI gavage study, which is described as having provided "positive evidence of hepatocellular carcinomas in mice," and (2) the 1985 NTP inhalation study, which the Addendum describes as "demonstrating that PCE can induce carcinogenic effects in both rats and mice through inhalation exposure."

Both the 1977 and 1985 studies have been severely criticized by the scientific community. Shortly after the 1977 gavage study was completed, NCI's own advisory committee, the Clearinghouse on Environmental Carcinogens, expressed serious reservations about the study as to both mice and rats. Clearinghouse members characterized the study as "poorly-designed," primarily because the animals received toxic doses of PCE. In its official statement, the Clearinghouse observed: "the significance of the bioassay . . . is significantly blunted by the evidence of toxicity in both the low and high dose groups," and noted that the effects observed with both rats and mice were "prima facie evidence for dose schedules in excess of the maximum tolerated dose." See transcript of proceedings of National Institutes of Health Clearinghouse on Environmental Carcinogens, Data Evaluation/Risk Assessment Subgroups, September 26, 1977 at 144.

Thus, NCI's own advisory committee (the Clearinghouse), as is abundantly clear from the transcripts of the relevant Clearinghouse meetings, long ago repudiated the 1977 bioassay as reliable evidence of carcinogenicity in either species.

NTP's conclusion that the 1985 inhalation study demonstrated "clear evidence" of carcinogenicity in both rats and mice has been similarly questioned. Dr. Robert A. Squire, previous Director of the animal bioassay cancer testing program at the National Cancer Institute, has reviewed NTP's report, including an on-site review of tissue slides from the test animal. Dr. Squire has noted several factors which cast doubt on NTP's finding of "clear evidence" of carcinogenicity in male rats and "some evidence" in female rats:

- o There currently are no generally accepted criteria for staging leukemias in rat. The ad hoc criteria used in the NTP study neither were reviewed in advance by experts in the field nor were similar to criteria used for staging leukemias in other species.

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- o There was an abnormally high incidence of leukemia in control animals, indicating some other environmental factor which affected the observed leukemia rate in test animals.
- o The leukemia finding is of doubtful relevance to potential effects of PCE on humans. The observed incidence of leukemia in rats is "a questionable endpoint for predicting human risk. There is no comparable human disease."
- o The finding of kidney tumors in male rats was not statistically significant and, in any case, occurred only in conjunction with nephrotoxic doses of PCE.

Dr. Squire also questioned the relevance of the NTP's findings of increased mouse liver cancers to humans. He noted the widely-recognized susceptibility of mice to develop cancer when exposed to substances which are also toxic to the mouse liver. For this reason, Dr. Squire concluded:

The mouse liver is generally not an appropriate surrogate for carcinogenesis risk in humans particularly where (like PCE) the compound tested is non-genotoxic and is administered at toxic levels.

Additionally, Clement Associates, Inc., in an audit of the 1985 NTP study, also identified a number of deficiencies. These included deficiencies in animal handling and identification, as well as general problems with the testing laboratory serious enough to require corrective action by NTP. Clement also expressed concerns similar to Dr. Squire's about the relevance of observed findings of rat leukemias, particularly in view of the arbitrary staging criteria used by NTP, and about the significance of the rat kidney tumors. Indeed, Clement concluded that the observed mononuclear cell leukemia in rats was unrelated to PCE exposure.

Significantly, the Science Advisory Board's Environmental Health Committee, Halogenated Organics Subcommittee, at its May 1986 review of the 1985 NTP findings concluded that all of the 1985 rat data, including the finding of leukemia in females, must be considered at best equivocal and is of doubtful relevance to humans.

The SAB Subcommittee in fact rejected all the evidence cited in the Addendum in support of Group B2 classification for PCE, except the findings of mouse liver tumors. While the Subcommittee accepted the 1985 positive findings in mice, it had reservations about whether those findings reflected a true carcinogenic mechanism. Regardless of the Subcommittee's reservations, these findings clearly do not represent "sufficient evidence" of carcinogenicity in animals under EPA's guidelines. Evidence is considered sufficient only if an increased incidence of tumors is found

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(a) in multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset.

49 Federal Register 46294, 46300 (November 23, 1984). Therefore, for the 1985 mouse liver tumor data to be considered sufficient evidence, it would have to be corroborated by at least one other test utilizing the same species or strain of mouse or utilizing another species. As noted, the 1985 study yielded no valid findings with regard to rats. The 1977 NCI study was repudiated by NCI's own advisory committee for use of doses exceeding the maximum tolerated dose in both species.

Even assuming NCI's 1977 findings of increased mouse liver tumors were reliable, this would still not be "sufficient" evidence. Positive findings of liver tumors in the B6C3F1 mouse in two (or more) experiments cannot be viewed as more than "limited" evidence of carcinogenicity.

The clear weight of scientific authority is against the use of the mouse liver tumor as a virtual sole indicator of effects in humans. Moreover, the species-specific effect of PCE on mice is well-documented. When NTP repeated the 1977 NCI gavage bioassay in 1983, it attempted to prove that liver damage from PCE is not a necessary precursor of liver tumors in the B6C3F1 mouse. In fact, the data demonstrated a contrary proposition--that hepatotoxicity is a prerequisite for hepatocarcinogenicity in this strain.

In mice, tetrachloroethylene was carcinogenic to the liver, but only at levels which were hepatotoxic, indicating that there are critical levels of cellular damage which may be necessary before there is an increase in cancer risk. This clearly has implications for human risk assessment and one may conclude that no-effect or threshold levels would probably also exist in man where there is no hepatotoxicity.

These findings are consistent with the absence of a genetic mechanism for PCE-induced mouse liver tumors. Dr. Marvin Kuschner, Dean of the School of Medicine at the State University of New York at Stonybrook, and a former member of the SAB Subcommittee on Airborne Carcinogens, as early as 1981 hypothesized that PCE is a liver toxin which, in the B6C3F1 mouse, damages liver cells. Again, under this theory, exposure to PCE at levels below that which destroys liver cells would not result in tumors in mice or other species.

More recently, researchers have discovered a cellular oncogene in spontaneous liver tumors in B6C3F1 mice. This finding also "supports the concept that the B6C3F1 mouse is hypersusceptible to liver development" and indicates that this animal "is dissimilar to the genetically diverse human population in its ability to activate, with a very high frequency, a specific tumor-associated oncogene." T. Fox & P. Watanabe, Detection of a Cellular Oncogene in Spontaneous Liver Tumors of B6C3F1 Mice, 228 Science at 596-97 (1985). This dissimilarity to humans militates strongly against reliance on liver tumors in this strain of mouse as a predictor of human carcinogenicity of PCE or, indeed,

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any other substance.

In recognition of these severe limitations, EPA's guidelines specifically provide that mouse liver-only tumor responses, even if replicated, should not be accorded the same weight as positive findings in multiple experiments with some different species. Thus, the guidelines state:

Under specific circumstances, such as the production of neoplasms that occur with high spontaneous background incidence, the evidence may be decreased to "limited" if warranted (eg., there are widely diverging scientific views regarding the validity of the mouse liver tumor as an indicator of potential human carcinogenicity when this is the only response observed, even in replicated experiments in the absence of short term or other evidence).

49 Federal Register at 46300. Since there is no substantive short term or other evidence (eg., genotoxicity or mutagenicity) for PCE, the 1977 and 1985 mouse data is, at most, a severely limited indication of possible human carcinogenicity.

Telling evidence for the lack of human carcinogenicity comes from the available epidemiological evidence for PCE. With a unit risk factor (as calculated by ARB) of $31\text{--}144 \times 10^{-6}/\text{ppb}$, evidence of an increased risk should be strongly discernible in drycleaning worker cohorts, even those of small size, considering the typical levels of exposure in the range of 40-60 ppm which have existed for 40 years or more.

A unit risk factor of $31\text{--}144 \times 10^{-6}/\text{ppb}$ is equivalent to $0.031\text{--}0.144/\text{parts per million}$, and greater than unity for 50 ppm exposures, ie, in the range of 1.55--7.20/50 ppm for lifetime exposures. Even corrected for daily duration of exposure and working span factors, the risk factor is purportedly in the range of 0.26--1.20/50 ppm.

Significant deficiencies exist with most epidemiological studies which have been reported, to the point where critically-disabling flaws disqualify these studies as a possible predictor of potential human carcinogenicity.

Specifically, save for the retrospective cohort mortality study by NIOSH, no study has been done where exposure was known to be limited to PCE. In fact, each of these other studies has either specified or acknowledged exposure to multiple solvents, including carbon tetrachloride, petroleum solvents, and others. A further limitation of these studies has been an inability to identify work occupations, with the result that the cohorts have consisted of an unknown mixture of laundry plant workers, drivers, and other unexposed employees.

Stationary Source Division
Air Resources Board

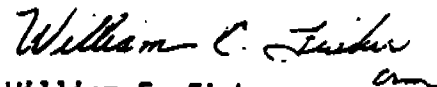
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While the full NIOSH cohort of 1,600 workers could not be fully identified as to solvent exposure, a subcohort of approximately one-third of the full group was known to have had only PCE exposures in their working careers. In the subcohort--the only PCE-specific exposure group ever studied--there was no overall increase of cancer, and no site-specific increase in cancer.

In conclusion, we believe that the equivocal bioassay data, coupled with the lack of any confirming human data where such would be expected, does not support a determination of potential/probable human carcinogenicity for PCE.

At the same time, our industry believes that until final evidence is available to establish an even-stronger determination of non-carcinogenicity, a continued effort to reduce in-plant exposures and environmental emissions represents the most prudent of courses. To that end, we offer a commitment to the Air Resources Board to work jointly in developing viable, innovative approaches to significantly reduce environmental emissions from our industry.

Sincerely,



William E. Fisher
Assistant General Manager/
Vice President

copy to: Caffey Norman
Earl Nichols, Paul Ceccarelli
Charles Riggott, Jon Meijer
Fax to G.Laumann

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County of San Diego



R. J. Sommerville
Air Pollution Control Officer

February 7, 1990

Robert Rood
Substance Evaluation Section
Air Resources Board
Attn: Perchloroethylene
P.O. Box 2815
Sacramento, CA 95812

**COMMENTS ON PROPOSED IDENTIFICATION OF PERCHLOROETHYLENE
AS A TOXIC AIR CONTAMINANT, PART A REPORT**

Even though the perchloroethylene usages in Table III-1 (Section III, page A-7) were directly referenced from Appendix A, these two sources do not agree and the difference is unclear. CARB predicts a proportional increase in dry cleaning emissions due to an increase in state population (Section III, page A-13). However, there is no mention whether tighter OSHA regulations in the work place and associated phasing-out of transfer systems were considered.

For dry cleaning operations, CARB uses an EPA quoted reduction value of 70% (Section III, page A-8) for facilities equipped with refrigeration units, while the San Diego Air Pollution Control District (APCD) has been using 90% as the accepted reduction value. This factor reflects control standards in current district rules and this discrepancy could mean an underestimation of perchloroethylene emissions from dry cleaning operations in San Diego County. Little mention was made of emissions from filter or distillate waste which has been shown to contribute significantly to overall perchloroethylene emissions.

The report gives the indication that throughout the state degreasers are significant contributors to perc emissions. San Diego County currently has approximately 20 of the estimated 350 perchloroethylene degreasers in California (Section III, page A-9) and the emissions are estimated from inventories to be 30 tons per year. This means that degreasers may not contribute to perc emissions in some districts as significantly as in others.

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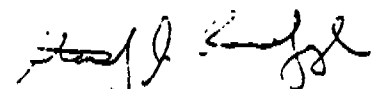
AIR POLLUTION CONTROL DISTRICT
9150 Chesapeake Drive, San Diego, California 92123-1095

February 7, 1990

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Emissions from state-wide maskant operation were not mentioned as significant contributors, while operations in San Diego County may be relatively large emitters of perchloroethylene.

Thank you for the opportunity to comment.



STANLEY J. ROMELCZYK
Senior Air Pollution Control Engineer

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II. AIR RESOURCES BOARD STAFF RESPONSES TO COMMENTS ON PART A

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A. COMMENTS FROM CITIZENS FOR A BETTER ENVIRONMENT

1. Comment:

The Part A report does not adequately assess total public perchloroethylene exposures for all indoor environments. Specifically, Part A neglects the exposure of workers, consumers and others in indoor microenvironments such as hospitals, schools, offices, and workplaces. Exposure to a consumer at a dry cleaning counter should be assessed.

Response:

The database for indoor exposure assessment is very limited. The report emphasizes indoor monitoring studies that were designed to provide representative data for Californians. So far, these types of studies have concentrated mainly on residential environments.

The studies reviewed include ones in which 1.) only personal exposure data was collected, 2.) personal exposure data was collected and indoor and outdoor concentrations were measured, and 3.) only indoor concentrations were measured. Although studies discussed in the report do not specifically target consumers at dry cleaning counters, the personal exposure studies include workplace exposures and exposures in consumer-accessible environments. For example, in the Total Exposure Assessment Methodology (TEAM) studies conducted in 1987, several participants noted visits to dry cleaning shops among their personal activities.

In addition to the personal exposure studies, an Environmental Protection Agency (EPA) study of the indoor environments of different public buildings is reviewed in Part A (Wallace et al., 1987; Sheldon et al., 1988). These public buildings include a hospital, a school, two homes for the elderly, and several offices. None of these public buildings are located in California.

We note that the California Occupational Safety and Health Agency (Cal-OSHA) has the specific mandate to review and regulate workers' exposures and workplace air levels.

2. Comment:

Information should be added to assess the multiple routes of exposure from the different sources of perchloroethylene in indoor air.

Response:

Currently, we do not have specific data to breakdown the perchloroethylene sources of exposure in indoor air. However, in keeping with the California Health and Safety Code section 39660.5, we assessed exposure from indoor air and discussed the relative contribution of indoor air to total exposure based on the available data.

3. Comment:

The European data on perchloroethylene exposure by ingestion should be related to exposure in California by identifying the mechanisms by which perchloroethylene contaminates the food supply. Cancer risks associated with the ingestion of perchloroethylene in food should be presented in the report.

Response:

The European data do not furnish a sound basis for assessing perchloroethylene exposure by ingestion in California because: 1.) the data are incomplete and dated, and 2.) the difference in dietary habits between the European study group and Californians can not be adequately assessed.

Although exposure through media other than air is included when information is available, the purpose of the ARB's Part A report is to identify mechanisms by which persons are exposed to airborne toxic substances. The Part B report prepared by the Department of Health Services assesses the cancer risk based on the ARB's exposure information. Some airborne toxicants, especially those of a particulate nature, contaminate food or water by deposition, adsorption, or dissolution. Due to its volatility and relative insolubility in water, the contribution of airborne perchloroethylene to concentrations of the substance in food or water is expected to be negligible.

4. Comment:

The report should include exposure and cancer risk assessment for: 1.) average and maximum indoor air concentrations, and 2.) daily maximum, chronic, and worst-case exposures from the combination of air and other environmental media.

Response:

The revised Part A report includes available data on maximum indoor air concentrations. Also, the daily dose via indoor air inhalation includes 24-hour average maximum concentrations.

There are sufficient data on ambient concentrations and near-source emissions to assess the cancer risk for exposed populations. The cancer risk assessment is summarized in the Staff Report/Executive Summary and more fully described in the Part B report. However, the database for perchloroethylene exposure via environmental media other than air is very limited and is not necessary to support our recommendation that perchloroethylene be identified as a toxic air contaminant.

5. Comment:

Superfund Amendments and Reauthorization Act (SARA) Title III data for toxic air contaminants should be included in reports. Also, the geographical distribution of the SARA Title III emissions should be presented.

Response:

The ARB staff has not used information from the SARA Title III, section 313 database in toxic air contaminant identification reports for the following reasons: 1.) only those industries which manufacture, process, or use chemicals above specified thresholds are required to report, 2.) emissions from utilities, military installations, hazardous treatment plants, agriculture, and motor vehicles are not required to be reported, 3.) the database relies entirely on self-reporting, that is, industrial facilities estimate their own emissions, and 4.) the emissions reported by a facility are not audited.

Using a variety of information sources has enabled the ARB staff to develop more complete emissions inventories than the SARA Title III reports.

The information contained in the Federal SARA Title III section 313 database is available to the public through the following agencies:

Office of Hazardous Materials Data Management
Environmental Affairs Agency
P.O. Box 2815
Sacramento, California 95812
Phone: (916) 327-1848 or 327-1849,

Pollutant Characterization Section
Noncriteria Pollutant Programs Branch (MD-15)
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711
Phone (919) 541-5371

6. Comment:

In section IV C, Exposure to Perchloroethylene Near Emission Sources, Part A should include the individual and population cancer risk for the population exposed to emissions from the eight South Coast facilities modeled by the ARB staff.

Response:

The individual and population cancer risk for the population surrounding the eight South Coast facilities is in the Staff Report/Executive Summary and the revised Part B report.

7. Comment:

Part A should include the emission levels for the eight South Coast facilities discussed in section IV C, Exposure to Perchloroethylene Near Emission Sources.

Response:

The revised report includes the estimated total emissions, as well as the range of emissions, for five facilities centered on the City of Industry and three facilities centered on Burbank.

B. COMMENTS FROM INTERNATIONAL FABRICARE INSTITUTE

1. Comment:

The results of a 1988 national survey by the industry indicate that 79 percent of all retail dry cleaners use perchloroethylene exclusively and 9 percent use perchloroethylene plus another solvent. California perchloroethylene-usage is probably the same or higher.

Response:

The information regarding the extent of perchloroethylene-usage by the dry cleaning industry is included in the revised Part A report.

2. Comment:

There are no known reasonable substitutes for perchloroethylene in the dry cleaning industry.

Response:

The purpose of the identification phase of the toxic air contaminant program is to determine whether or not a compound should be listed as a toxic air contaminant. If perchloroethylene is identified as a toxic air contaminant, the possibility of substituting perchloroethylene with another cleaning solvent will be considered in the risk management phase of the process.

3. Comment:

One example of emissions limitations is the range (0.35 to 1.5 pounds per day) in the South Coast Air Quality Management District's (SCAQMD's) Proposed Rule 223. This limitations range far exceeds that possible under Reasonably Acceptable Control Technology (RACT), Best Available Control Technology (BACT), or Maximum Available Control Technology (MACT). In fact, it is more stringent than the emissions limits achievable at the best operating plant in the nation by an order of magnitude or better.

Response:

The comment above should be directed to the SCAQMD. The Part A report provides exposure information for the proposed identification of perchloroethylene as a toxic air contaminant and does not propose or endorse any district control measure. If perchloroethylene is identified as a toxic air contaminant, all possible control measures will be considered in the risk management phase of the process.

4. Comment:

The ARB staff has overestimated the number of dry cleaning operations in California. The number of dry cleaners using perchloroethylene in California is approximately 2400.

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Response:

The revised Part A report states that there are approximately 3,000 dry cleaning operations in the state using perchloroethylene. Based on permitting records, the estimated total number of dry cleaners using perchloroethylene in just the Bay Area and South Coast air quality management districts is approximately 2600.

5. Comment:

In Appendix A page A-2, the ARB staff estimates that 18,100 tons/year of perchloroethylene are used by California dry cleaners based on sales by solvent producers. The perchloroethylene-usage by California dry cleaners is overestimated because solvent producers equate the sales of dry cleaning-grade perchloroethylene with sales to dry cleaners when, in reality, other businesses use this grade of the solvent.

Response:

The estimate in Appendix A used data from solvent producers for 1985 while the estimate in Chapter III of Part A (12,857 tons) was based on a survey of California halogenated solvent distributors for the 1987 inventory year. In order to avoid confusion, Appendix A (Methods for Estimating Usage and Emissions of Perchloroethylene in California) is not included in the revised Part A report. The methodology for estimating usage and emissions from each source is now included in the text of the report.

The 1987 estimate in the revised Part A report relies on the distributor's records of the establishments to which they sold perchloroethylene and not on the grade of perchloroethylene sold.

6. Comment:

The ARB staff use an overly high perchloroethylene emission factor of 0.88 pounds emitted for each pound used in dry cleaning.

Response:

The 0.88 emission factor for nation-wide dry cleaners developed by Wolf and Myers (1987) is based on subtracting the amount of solvent waste generated in 1984 from the total amount of perchloroethylene used that year. The difference is assumed to be perchloroethylene emissions. The ARB staff believes that the 0.88 factor is appropriate for estimating 1987 perchloroethylene dry cleaning emissions. Currently, the staff expects that overall perchloroethylene emissions from the California dry cleaning industry are decreasing due to: 1.) the adoption of dry cleaning control measures by several air pollution control districts, 2.) the institution of lower permissible exposure limits for workers by the California Occupational Safety and Health Agency (Cal-OSHA) in 1990, 3.) the adoption of DHS hazardous waste regulations requiring the storage of filter and still-bottom waste in air-tight containers, 4.) the trend to replace transfer units with

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dry-to-dry units, and 5.) the trend for large industrial cleaners to use soap and water rather than perchloroethylene. This information is included in the revised Part A report.

D. COMMENTS FROM SAN DIEGO AIR POLLUTION CONTROL DISTRICT

1. Comment:

The amount of perchloroethylene-usage shown in Table III-1, page A-7, does not agree with the amount shown in Appendix A, page A-1.

Response:

The perchloroethylene-usage shown in Table III-1 is based on a survey of halogenated solvent distributors for the 1987 inventory year while the usage shown in Appendix A is based on the amount of perchloroethylene shipped to California in 1985. To avoid confusion in the revised Part A report, the methods for estimating usage and emissions of perchloroethylene in California are described by source-category in the body of the text.

2. Comment:

On page A-13, the ARB staff predicts an increase in dry cleaning (and perchloroethylene emissions) due to a projected increase in California's population. Was the institution of tighter Cal-OSHA regulations in 1990 considered in this prediction?

Response:

The preliminary draft of Proposed Identification of Perchloroethylene as a Toxic Air Contaminant was issued before the Cal-OSHA permissible exposure limits of 25 parts per million (ppm) became effective in April 1990. The revised Part A report discusses the likely trend toward decreasing perchloroethylene emissions from dry cleaners (please see the response to comment B 6 above). In the revised report, the projected increase in population is linked to a predicted increase in perchloroethylene-usage and emissions for industries other than dry cleaning.

3. Comment:

On page A-8, the ARB staff uses an EPA estimate of 70 percent reduction in perchloroethylene emissions from dry cleaning facilities equipped with refrigerated condensers. Based on control standards in the San Diego Air Pollution Control District rule, the district estimates a 90 percent reduction in emissions. Is San Diego underestimating perchloroethylene emissions from dry cleaning in the county?

Response:

The San Diego rule specifies that "90 percent or more by weight of the halogenated organic compounds be removed by the device ..." but does not specify the type of air pollution control device to be used. As mentioned in Part A, carbon adsorbers (most often associated with transfer dry cleaning operations) are estimated to reduce

emissions by about 95 percent while refrigerated condensers (most often associated with dry-to-dry dry cleaning operations) reduce emissions by about 70 percent. In transfer operations, washed clothes are physically transferred to a drying unit, thus furnishing an additional opportunity for perchloroethylene emissions. In dry-to-dry operations, these emissions are avoided by washing and drying clothes in the same unit. The total reduction in perchloroethylene emissions using the dry-to-dry unit with a refrigerated condenser should be very close to that achieved using a transfer unit with a carbon adsorber. Both systems are capable of the 90% reduction specified by the San Diego District rule.

4. Comment:

In Part A, little mention was made of emissions from filter or distillate waste which has been shown to contribute significantly to overall perchloroethylene emissions.

Response:

Given the use of air pollution control devices such as carbon adsorbers and refrigerated condensers, filter and distillate residues may be a significant source of perchloroethylene emissions. Once the residues are removed to air-tight containers in accordance with hazardous waste regulations, they cease to contribute to dry cleaning emissions. This information is reflected in the revised Part A report.

5. Comment:

On page A-9, the Part A report indicates that degreasers are a significant source of perchloroethylene emissions throughout the state. Actually, degreasers may not significantly contribute to emissions in some districts. For example, San Diego has only 20 degreasers (out of California's estimated 350 degreasers) with estimated emissions of 30 tons per year.

Response:

Some districts are expected to have more degreasers and, therefore, more emissions from degreasing than other districts. Currently, there is insufficient data to estimate perchloroethylene emissions from degreasers in each district of California. Such information should become available as data from the AB 2588 Hot Spots Emissions Inventory are analyzed.

6. Comment:

Emissions from state-wide maskant operations were not mentioned as significant sources of perchloroethylene emissions. Such operations in San Diego County may be relatively large perchloroethylene emitters.

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Response:

The halogenated solvent distributors surveyed by the ARB staff could not identify the purchasers of 642 tons of perchloroethylene sold in the 1987 inventory year. An unknown amount of the 642 tons was expected to be used by the semi-conductor industry in maskant operations. Thus, in the Part A report, the perchloroethylene emissions from maskant operations are included in the emissions attributed to the Miscellaneous source-category.

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III. DEPARTMENT OF HEALTH SERVICES STAFF RESPONSES TO COMMENTS ON PART B

DEPARTMENT OF HEALTH SERVICES
STAFF RESPONSES TO PUBLIC COMMENTS
ON THE JANUARY 1989 REVISION OF PART B
OF THE DRAFT REPORT TO THE AIR RESOURCES BOARD
ON TETRACHLOROETHYLENE (PCE)

General Comments

1. Comment: The statement in Chapter 1 of the draft document that PCE is used for coffee decaffeination is incorrect (Halogenated Solvents Industry Alliance, February 1, 1990 [HSIA], page 27).

Response: The reference to use of perchloroethylene in decaffeination of coffee was eliminated.

2. Comment: Chapter 1 of the draft document states that the Occupational Safety and Health Administration permissible exposure limit (OSHA PEL) for PCE is 50 ppm for an 8-hour time-weighted-average (TWA). The OSHA PEL has recently been changed to 25 ppm for an 8-hour TWA (HSIA, p. 27).

Response: The revised PEL has been added to the document.

Topic: Hazard Identification

1. Comment: The weight of available scientific evidence does not support the conclusion that PCE is a probable human carcinogen (HSIA, p. 4). Significant questions about the weight of evidence for possible human carcinogenicity of PCE indicate that designation of PCE as a carcinogenic air pollutant or as "a 'potential' (i.e., probable)" human carcinogen is unwarranted (International Fabricare Institute, January 31, 1990 [IFI], pp. 3, 5-10).

Response: Experimental studies have indicated that PCE or its metabolites are genotoxic and can produce cancer in laboratory animals. PCE induced DNA strand breaks in liver and kidney cells of mice treated in vivo. PCE induced transformation of rat embryo cells. It induced sex-linked recessive, lethal mutations in Drosophila. PCE induced gene conversion and mitotic recombination in yeast. PCE has been shown to be mutagenic to plants in vitro. When mice were exposed to PCE by oral or inhalation administration, it produced hepatocellular carcinomas. Exposure of rats by inhalation to PCE produced an increased incidence of leukemias. Since PCE is genotoxic and can produce cancer in laboratory animals, it should be considered a probable human carcinogen.

2. Comment: Applying the criteria of IARC (the International Agency for Research on Cancer) does not justify hazard identification premised on a determination that it is more likely than not that PCE poses a human cancer risk (HSIA, p. 21).

Response: As indicated by IARC's preamble to Supplement 7, several agents exhibited evidence of carcinogenicity in experimental animals prior to evidence being obtained from epidemiological studies or case

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reports. There are 44 agents for which there is sufficient or limited evidence of carcinogenicity to humans and all 37 that have been tested adequately experimentally produce cancer in at least one animal species. And as stated in IARC (1987) "although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, nevertheless, in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans." Consequently, since there is sufficient evidence of carcinogenicity in experimental animals for PCE, it is considered a probable human carcinogen. This is indicated in the IARC monographs on the evaluation of carcinogenic risks to humans Supplement 7 (1987).

3. Comment: Chapter 1 of the draft report states that the EPA is likely to classify PCE in its risk assessment category B2, but EPA staff have indicated that the Agency has not reached a final decision on classifying PCE. The EPA's Science Advisory has placed PCE "on the continuum between" categories B2 and C. This conclusion should be reflected throughout the document wherever EPA's classification of PCE is mentioned (HSIA, p. 20).

Response: Perchloroethylene is currently classified as a B2 carcinogen by EPA. Discussions with EPA staff indicate that the agency still supports the B2 classification and has made regulatory decisions based on this determination. For example, in January 1991 EPA set PCE drinking water standards based on the agency's position that PCE is a probable (B2) human carcinogen. However, it is likely that this compound will undergo continual review at EPA.

4. Comment: California's carcinogen risk assessment guidelines ("Guidelines for chemical carcinogen risk assessments and their scientific rationale," State of California, Health and Welfare Agency, Department of Health Services, November 1985) suggest that PCE should not be identified as posing a cancer hazard to humans (HSIA, pp. 21-22).

Response: As indicated in California's Carcinogen Risk Assessment Guidelines, the document on the Health Effects of Tetrachloroethylene does discuss the animal bioassays of cancer in detail. The document also discusses the pharmacokinetics and other factors pertaining to the potential genotoxicity and carcinogenicity of PCE. The guidelines also indicate that IARC follows all of the suggested procedures necessary to properly identify a chemical as a potential human carcinogen. The uncertainties of evaluating PCE as a carcinogen are discussed throughout the chapter on toxic effects in animals. The uncertainties in the pharmacokinetic and metabolism data are also discussed in the document. It is the conclusion of DHS staff that the overall weight of evidence regarding PCE indicates that it is a potential carcinogenic hazard to humans.

5. Comment: The mixed results of the in vitro genotoxicity studies of tetrachloroethylene oxide mentioned on page 3-22 of the draft document should be interpreted cautiously. The Halogenated Organics Subcommittee of the EPA Science Advisory Board has noted that PCE is not mutagenic,

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and that tetrachloroethylene oxide is not a demonstrated metabolite of PCE and is apparently not carcinogenic (HSIA, pp. 18-20).

Response: The determination that PCE as a genotoxic agent is not based solely on the genotoxicity of tetrachloroethylene oxide. It is simply indicated in the document that in addition to some positive genotoxic studies on PCE itself, mutagenic activity has been determined from PCE metabolites.

6. Comment: Overall, the draft document's evaluation of the epidemiological data is consistent with that of EPA's Science Advisory Board. Since that Board's review, results have become available from the most complete epidemiological study of the industry conducted to date [Brown DP and SD Kaplan (1987) Retrospective cohort mortality study of dry cleaner workers using perchloroethylene. J Occup Med 29:535] (HSIA, p. 17). This study shows no increased risk of cancer in dry cleaning workers exposed only to PCE (HSIA, p. 18, IFI, pp. 9-10). This was the only group ever studied where exposure was known to be limited to PCE (IFI, pp. 9-10).

Response: As indicated in the health effects document, there is inadequate evidence of carcinogenicity in humans. This conclusion is based on a number of epidemiologic studies including the study of Brown and Kaplan.

Topic: Dose-Response Assessment

1. Comment: The draft report should be revised to reflect more clearly the qualitative differences in metabolism of PCE among rodent species and humans (HSIA, p. 4).

Response: The differences in metabolism of PCE are discussed in Section 2 on pharmacokinetics and metabolism as well as the Sections on quantification of PCE's carcinogenic potency. In addition, numerous citations are given to other authors that have also reviewed the metabolic data on PCE. Furthermore, in the revised risk assessment of PCE, the differences in metabolism among rodents and humans are taken into account through use of a pharmacokinetic model.

2. Comment: If the final report includes an estimate of potential risk, it should incorporate all available scientific data. Pharmacokinetic information is not reflected in the draft document. The draft document is therefore inconsistent with California's carcinogen risk assessment guidelines. In light of this and the availability of extensive pharmacokinetic data, the rejection of a physiologically-based pharmacokinetic model in favor of an approach based on default assumptions appears to be an abuse of DHS' discretion (HSIA, pp. 4-5, 24).

Response: The document has been revised along the lines of HSIA's comments. That is, pharmacokinetic data which were incorporated in the document in the previous draft had been revised with additional

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pharmacokinetic information. This has resulted in a 15-fold lower estimate of upperbound risk. A risk estimate based on pharmacokinetic information was used in the current document to establish the range of risks and the best value for the upper bound of risk.

3. Comment: Page 5-2 of the draft document notes that using the metabolized dose to calculate cancer potency would result in a higher potency value than would using the administered dose. This is mathematically correct but misleading and irrelevant. Metabolized doses will lead to higher potency values, but equivalent doses will be smaller. Risk estimates themselves can be higher or lower depending upon the metabolism and distribution of the particular compound (HSIA, p. 27).

Response: The statement regarding the higher potency for metabolized dose is given for information to the reader. Since there has been discussion in the scientific literature indicating that pharmacokinetic doses may imply a lower risk to humans, the reader might be confused seeing higher potency values in the document. Consequently, the statement regarding higher potency value will remain in the document in order to clearly state to the reader that the potency values for the metabolized dose throughout the document may be higher than those for the applied dose. As indicated in the comment, in terms of actual risk to humans, the use of a pharmacokinetic dose does not itself imply that there will be higher or lower risk to humans. Instead, the risk estimate will depend upon the model chosen, the parameters used, and the assumptions made regarding the parameters and the model.

4. Comment: The statement on page 1-9 of the draft document that range of potencies reported by EPA (2.9 to 9.5×10^{-7}) includes the DHS range is incorrect (HSIA, p. 27).

Response: The range of unit risks reported in the Health Effects document on tetrachloroethylene is 3 to 106×10^{-7} per $\mu\text{g}/\text{m}^3$ of PCE. This range includes the potencies reported by EPA.

5. Comment: There is no good scientific basis for risk estimates, based on default assumptions, that are 20-fold higher than those being considered by EPA (HSIA, p. 22).

Response: Instead of using the default assumptions, the range of risk reported in the document now incorporates the pharmacokinetic information. Consequently, the previous risk estimate was lowered 15-fold.

6. Comment: The gavage bioassay reported by the National Cancer Institute in 1977 does not appear to be a "properly conducted bioassay" as required by the California carcinogen risk assessment guidelines (HSIA, p. 6).

Response: The quality of the NCI 1977 study is discussed extensively in Section 3 and Section 5 of the document on the Health Effects of Tetrachloroethylene. Due to various shortcomings in the study, the risk

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estimate from that study is not included in the range of risk or the best value for risk recommended by the Department.

7. Comment: Much relevant research is neither discussed nor referenced in the draft document (HSIA, p. 16). [The commentor submitted copies of the following articles: (1) Brown DP and SD Kaplan (1987) Retrospective cohort mortality study of dry cleaner workers using perchloroethylene. J Occup Med 29:535. (2) Elcombe CR (1985) Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: a biochemical human hazard assessment. Arch Toxicol Suppl 8:6. (3) Odum J, T Green, JR Foster and PM Hext (1988) The role of trichloroacetic acid and peroxisome proliferation in the differences in carcinogenicity of perchloroethylene in the mouse and rat. Toxicol Appl Pharmacol 93:103. (4) Herren-Freund SL, MA Pereira, G Olsen and AB DeAngelo (1986) The carcinogenicity of trichloroethylene (TCE) and its metabolites, trichloroacetic acid (TCA) and dichloroacetic acid (DCA), in mouse liver. Proc Amer Assoc Cancer Res 27:91, abstr. 356. The document should address several recently published works that apply physiologically-based pharmacokinetic (PB-PK) models. (HSIA, pp. 23-25).

Response: The study by Brown and Kaplan is discussed in the health effects document in Section 4 under "Epidemiologic Evidence for Carcinogenicity in Humans." The study of Elcombe (1985) deals with TCE and is referred to in Section 3 under the subheading "Hepatic Toxicity." The work of Odum et al. (1988) was added and is discussed in Section 3 under the subheading "Hepatic Toxicity." The abstract of Herren-Freund et al. (1986) has not been added to the document, however, an article by the authors with the same title published in 1987 has been incorporated into Section 3 under the subheading "Tests for DNA or Chromosomal Damage". Studies applying PB-PK models for PCE have been discussed in the revised Health Effects document and pharmacokinetic information has been incorporated in estimating carcinogenic risks.

8. Comment: Although it true that PB-PK models do not account for inter-individual variability [this is stated on page 5-51 of the draft document], the same statement could be made for other models, including the LMS [linearized multistage model], unless the surface area factor is viewed as an individual tissue sensitivity factor (HSIA, p. 28).

Response: The standard approach used in risk assessment by EPA and DHS incorporate assumptions that would take into account inter-individual variability. That is, the use of an applied dose and the linearized multistage model are health protective assumptions which have a scientific basis. These health protective assumptions were made to incorporate potential inter-individual variability within the human population. Consequently, when a new procedure is suggested to replace an existing procedure, questions regarding incorporations of factors that protect certain populations need to be considered. The commentor compares the linearized multistage model with the pharmacokinetic model. This is an inappropriate comparison in that use of a pharmacokinetic model does not preclude use of the linearized multistage model as well. In the DHS document both models are used together. Instead, the comparison should be made between use of the pharmacokinetic model and use of an applied dose model. The current perchloroethylene document

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uses a pharmacokinetic model and an estimate of the uncertainty to account for population differences in metabolism. In this way, inter-individual differences can be taken into account. The surface area correction is used to consider the differences in sensitivity of tissue response between rodents and humans.

9. Comment: Use of body-surface area correction factors is not appropriate in the case of PCE; body weight provides a better basis for dose adjustment (HSIA, p. 23).

Response: The use of a surface area correction factor in the adjustment of rodent risk to human risk results in a difference of rodent to human risk of less than three fold. The use of the surface area correction factor is discussed extensively in Section 5 of the risk assessment document. In short, the surface area correction factor is used in order to take into account interspecies differences in tissue response to perchloroethylene.

10. Comment: The q^* correction on the bottom of page 5-8 should be clarified to differentiate between exposure period and observation period. If the dosing is for only one year but the animals are observed for an additional year, and the natural lifespan of mice and rats is assumed to be two years (104 weeks), the correction factor should be $104/T_e$ raised to the unity power, not the third power (HSIA, p. 28).

Response: The recorection refers to a shortened lifespan resulting in a shortened exposure. This has been clarified in the document. The correction referred to in the comment is an adjustment for shortened exposure as described in Section 5 under the topic of "Dose Adjustments". For this correction the length of the exposure, L_e , is simply divided by the lifespan of the animal.

11. Comment: The range of unit risk estimates presented in the draft document is inconsistent with human experience. Calculations with these unit risk estimates for persons occupationally exposed to PCE yield very large, very detectable, potential risks, and indicate that the unit risk estimates vastly overestimate the potential risks to humans (HSIA, pp. 25-26, 29; IFI, p. 9).

Response: A number of epidemiologic studies have been conducted on workers most likely exposed to perchloroethylene (PCE), those working in the dry-cleaning industry. In these studies it is not known how often they were exposed to PCE or the concentrations to which they were exposed to. Furthermore, confounding factors such as low economic status, smoking, alcohol use, and exposures to other carcinogenic solvents, make it difficult to link human exposure to PCE with cancer. This is discussed in Section 4 under the heading "Epidemiologic Evidence for Carcinogenicity in Humans."

One of the more complete studies was conducted by Brown and Kaplan (1987). In this study, exposures ranged from 3 to 22 ppm (i.e., 12.5 ppm average). There were 1,690 workers in this study with 619 workers primarily exposed to PCE. Assuming a length of employment of 5 years, the lifetime average exposure would be 0.21 ppm

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((5/7)(8/24)(5/70)(12.5)=0.21). Multiplying the risk times the exposure and the number of persons in the study ((1690) x (0.21 ppm) x (56 x 10⁻³)(ppm)⁻¹ = 20 indicates that an increase of 20 cancers would be expected. In the study there were 142 observed cancer deaths while only 123 were expected, i.e., an increase of 19. Thus, the estimate is close to the value expected.

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