HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE

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

SEP 25 1987

September 22, 1987

FILING INSTRUCTIONS:
W/HJ
Trichloroethylene
EPA HAD Addendum
HSIA Comments to EPA
KEYWORDS:

TO: HEALTH AND SCIENCE COMMITTEE
METHYLENE CHLORIDE TASK FORCE
TRICHLOROETHYLENE TASK FORCE

HAD ADDENDA COMMENTS

Attached are HSIA's final comments to EPA on the Addenda to the Health Assessment Documents for methylene chloride and trichloroethylene.

Daniel M. Byrd III
Director of Scientific Affairs

Attachments

SL 037884

HSIA HALOGENATED SOLVENTS INDUSTRY ALLIANCE

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

September 17, 1987

Project Officer for Trichloroethylene [C]
Office of Health and Environmental
Assessment (RD-689)
U. S. Environmental Protection Agency
Room 3812
Waterside Mall
401 M Street, S.W.
Washington, D. C. 20460

Re: External Review Draft Addendum to the Health Assessment Document for Trichloroethylene: Updated Carcinogenicity Assessment, EPA/600/8-82/006FA

Dear Sir:

The Halogenated Solvents Industry Alliance (HSIA) offers the enclosed comments on the Addendum to the Health Assessment Document for Trichloroethylene, 52 Federal Register 27257 (July 20, 1987).

HSIA is an association of producers, distributors, importers, and users of halogenated solvents, including trichloroethylene. Our members, as well as other users of trichloroethylene, have a vital interest in the accuracy and scientific validity of the Addendum as it is likely to be widely distributed and applied in a number of contexts at the federal, state, and local levels.

Sincerely,

Paul A. Cammer

Paul A. Cammer, Ph.D.
President

Enclosure

SL 037885

BEFORE THE
U. S. ENVIRONMENTAL PROTECTION AGENCY

External Review Draft Addendum to the Health Assessment Document for Trichloroethylene: Updated Carcinogenicity Assessment EPA/600/8-82/006FA

Comments of the
Halogenated Solvents Industry Alliance
1225 19th Street, N.W.
Suite 300
Washington, D. C. 20036

Paul A. Cammer, Ph.D.
President
September 16, 1987

Of Counsel:
W. Caffey Norman, III
Herron, Burchette, Ruckert
& Rothwell
1025 Thomas Jefferson Street, N.W.
Washington, D. C. 20007

Introduction

EPA's announcement of the availability of an External Review Draft of the Addendum to the Health Assessment Document for Trichloroethylene requested public comments.[1/] The Halogenated Solvents Industry Alliance (HSIA) offers these comments on EPA's assessment of the carcinogenicity of trichloroethylene. HSIA is an association of producers, distributors, importers, and users of halogenated solvents. Our members, as well as other users of trichloroethylene, have a vital interest in the accuracy and scientific validity of the Addendum.

Overall, the draft Addendum reaches erroneous conclusions about the potential human carcinogenic effects of trichloroethylene. These apparently occur because the draft Addendum does not make a serious effort to analyze some crucial evidence, such as epidemiological studies, and because exclusive weight is placed on certain theories regarding mechanism of action. In some cases reasonable alternative theories have not been addressed, and in others the proposed explanations are untenable. Because the final Addendum may form the basis of various EPA regulatory efforts (e.g. air, water, solid waste, toxic substances) as well as stimulate initiatives by federal, state, and local levels of government, the draft should be revised and resubmitted for public comment. The most serious deficiencies are summarized below.

(1) EPA has not carried out an adequate evaluation of a new, important epidemiology study by Shindell and Ulrich, which has a sufficiently large cohort to detect a risk double that of background. Neither the Shindell and Ulrich study nor the six other epidemiology studies support the conclusion that human exposure to trichloroethylene increases cancer risk. EPA has not compared the projected evidence to the statistical limits of risk detectability based on the negative human studies.

(2) Animal evidence is "limited" under EPA's guidelines for carcinogen risk assessment because trichloroethylene does not elicit a strong tumor response at any site and because of metabolic evidence that trichloroethylene induced mouse liver tumors are not indicative of human risk.

(3) The data on metabolism do not support the theory that the total amount of metabolites relates to biological effects. In contrast, production of one metabolite, trichloroacetic acid, has been shown to be causally related to the occurrence of mouse liver tumors.

(4) Trichloroethylene lacks genotoxicity for mammals and other higher animals.

(5) The draft Addendum is organized around a chapter titled "oncodynamics," which actually represents an amalgam of pharmacokinetic and mechanism-of-action interpretations. EPA should divide this chapter into two, one each for pharmacokinetics and mechanism of action and present this topic in a more understandable fashion.

If the Office of Research and Development were to carry forward the present draft's erroneous interpretation of the tumor data, a serious problem of research disincentive would occur. HSIA member companies have devoted considerable resources to the development of mechanism of action information about the induction of mouse liver tumors, which the draft Addendum does not utilize. Instead it relies on mouse lung tumors, rat Leydig cell tumors, and rat leukemias, all of highly uncertain significance or relevance to man. While scientists are addressing these data, this work will go slowly, particularly because the reported rat tumors occur at low frequency and do not appear reproducible in different laboratories. Motivation to pursue this work will be lacking if EPA does not utilize the existing data.

Epidemiology Studies

The draft Addendum indicates that EPA considers the epidemiologic data base inadequate to evaluate the carcinogenic potential of trichloroethylene. Epidemiology studies cannot generally disprove a cancer risk estimate based on animal studies, because the exposures will be lower and because experimental conditions cannot be controlled. Nevertheless, the 1985 Health Assessment Document (HAD) summarizes six such studies on trichloroethylene, none of which indicated elevated levels of cancer deaths. These studies have been criticized by EPA, but, as the 1985 HAD states (p. 8-128), none is consistent with or supports the hypothesis that trichloroethylene is a human carcinogen:

> The epidemiologic studies on TCI [trichloroethylene] provide no evidence for its carcinogenicity...

The draft Addendum does not take into account the available human epidemiology evidence. The best scientific evidence addressing the question of human carcinogenicity of any substance is provided by epidemiology studies. Such studies are often confounded by factors such as cohort size, extent of exposure,

latency considerations, and comparison to appropriate control cohorts, which frequently result in the inability to prove an absence of hazard.

There is, moreover, a subsequent significant addition to the epidemiological data base for trichloroethylene: a study by Shindell and Ulrich of a cohort of over 2,600 employees who worked three months or more during the period from January 1957 to July 1983 in a manufacturing plant that used trichloroethylene as a degreasing agent.[2/] Ninety-eight percent of the cohort was traced, accounting for over 16,000 man-years of employment (i.e., an average of over 6 years per employee) and over 38,000 man-years of follow-up. This study found no increased risk of cancer in the exposed population. Rather, the study found a deficit of cancer-related deaths compared to the number expected in exposed white male workers, and this result was statistically significant ($p < 0.05$). This study offers a far more extensive period of follow-up and a larger cohort than previous studies, and thus adds substantially to the scientific data base concerning human health effects. The occupational exposures in the study, particularly in the past, were orders of magnitude higher than ambient exposures.

The review of the Shindell and Ulrich study in the draft Addendum does not adequately assess the utility of these data to assist in a determination of carcinogenic risk of trichloroethylene exposure to humans. The study follows rather standard cohort analysis procedures. While the study does not determine the exposures which the cohort would have received historically, it indicates that monitoring of current employees suggests that exposures were within the OSHA Permissible Exposure Limit of 50 ppm. More detail on exposure would be desirable, but this does not preclude a reasonable assessment of the mortality experience of the cohort in order to determine if there is an increased risk of cancer in this population.

In short, the evaluation of the Shindell and Ulrich study in the draft Addendum is inadequate. The draft Addendum tends to focus on the presentation rather than on the strength of the study to detect an effect. There should be an evaluation of the power of the study, i.e., its ability to detect an increased risk of cause-specific mortality when one exists in the cohort. Power calculations for various detectable risks in the Shindell and Ulrich study are presented below for all cancers, nonrespiratory cancer, and respiratory cancer.

Power

Relative Risk	All Cancers	Nonrespiratory Cancer	Respiratory Cancer
1.5	80.2%	62.0%	44.6%
2.0	99.8%	97.6%	86.9%
3.0	100.0%	100.0%	99.9%

These calculations suggest that this study cohort was of a sufficient size and the age and follow-up were adequate to detect a 50% increased risk of death due to cancer in this working population, if in fact one existed. As the Agency tends not to require interspecies site concurrence for cancer, the "all cancers number" is the most useful. It demonstrates that this study has very good sensitivity for an epidemiology study. The power of the study to detect increased risk of nonrespiratory cancer and respiratory cancer is also quite good, demonstrating that a two-fold risk could have been detected 90% of the time.

The 1985 HAD reports a comparison of an upper confidence limit from a study by Axelson and coworkers to the plausible upper bound estimated from animal studies. While this is a valid comparison, EPA should prepare estimates from the animal data using similar methods (but including dose correction) to those used for the human epidemiology estimate. Even better, EPA could apply the multistage model to human data, preferably to the Shindell and Ulrich cohort with exposure estimates based on ranges for the occupational categories.

Given the power of the Shindell and Ulrich study, the failure of the draft Addendum to use it to calculate an upper bound on risk is a major shortcoming in the document. It is not appropriate to continue to rely on the upper bound computed in the 1985 HAD from the Axelson study, which was far less comprehensive.

Interpretation of Animal Studies

The animal bioassay results are summarized below.

I. Mouse Studies

A. 1976 NCI Study

This gavage study showed an increased incidence of liver tumors in male and female $B_6C_3F_1$ mice.[3] Science Advisory Board members and others have offered a number of criticisms of the study, including the use of massive doses in large amounts of corn oil and improper housing of the test animals.[4] Interpretation of the study is confounded by the presence of stabilizers, including epichlorohydrin, a rodent carcinogen, in the test material. As noted in the draft Addendum, a large variation in response occurred between male $B_6C_3F_1$ mice from two suppliers, and it is appropriate to eliminate these responses from consideration.

B. 1982 NTP Study

This gavage study also showed an increased incidence of liver tumors in $B_6C_3F_1$ mice.[5] Although the test material was purified, the same gavage dosing technique was used as in the 1976 study, resulting in bolus administration of the dose, which seriously alters the absorption, pharmacokinetics, and metabolism of the test material relative to human exposure conditions.

C. 1980 Henschler Study

NMRI mice were exposed to purified trichloroethylene by inhalation.[6] No increase in cancer was reported in male mice. An increase in the incidence of lymphomas was observed in females, but the authors did not ascribe this effect to trichloroethylene exposure.

D. 1984 Henschler Study

This corn oil gavage study showed no significant increases in tumors in either sex of Swiss mice gavaged with purified trichloroethylene.[7] The authors concluded that "there is no indication of a tumorigenic potential of pure, amine based-stabilized TRI [trichloroethylene]" and "this study does not support the suggestion that trichloroethylene itself is carcinogenic under realistic exposure conditions."

E. 1983 Fukuda Study

ICR mice were treated by inhalation with trichloroethylene. No increased incidence of tumors was observed in the males.[8] Female mice had an increased incidence of lung tumors at the two highest doses. If adenomas and carcinomas are combined, no increase in tumors was observed. Instead, there was a high incidence of benign adenomas in untreated controls, some of which appeared to convert to adenocarcinomas after exposure to trichloroethylene.

F. Maltoni Study

This inhalation study showed an increased incidence of lung tumors in male Swiss mice and female $B_6C_3F_1$ mice.[9] Only benign adenomas were found, not carcinomas. Hepatomas also were observed in male Swiss mice and both sexes of $B_6C_3F_1$ mice. These results have not been peer-reviewed.

G. Van Duuren Study

This series of studies in female Swiss mice involved administration of trichloroethylene by topical, subcutaneous, and gavage routes and included initiation-promotion studies.[10] No carcinogenic response was reported.

H. Herren-Freund Study

No statistically significant increase in tumors was found in male $B_6C_3F_1$ mice exposed to trichloroethylene by drinking water in this study.[11] Treatment by a trichloroethylene metabolite increased the incidence of liver carcinomas.

II. Rat Studies

A. 1976 NCI Study

This gavage study showed no increased incidence of cancer in Osborne-Mendel rats.

B. 1982 NTP Study

This gavage study in Fischer 344 rats was considered inadequate to evaluate the presence or absence of a carcinogenic response.

C. 1987 NTP Study

These gavage studies in four strains of rats were considered inadequate to evaluate the presence or absence of a carcinogenic response.[12]

D. 1980 Henschler Study

This inhalation study showed no increased incidence of cancer in Wistar rats.

E. 1983 Fukuda Study

This inhalation study showed no increased incidence of cancer in female Sprague-Dawley rats.

F. 1986 Maltoni Studies

These studies in Sprague-Dawley rats, both by gavage and inhalation, reportedly showed an increased trend or incidence of leukemias, renal adenocarcinomas and Leydig cell tumors in males. No increased incidence of cancer was observed in the females. As noted in the draft Addendum, the response rate for r nal tumors was low. These results have not been peer-reviewed. Additionally, the incidence of "leukemias" is within the range of values for control groups reported in other Maltoni studies.

III. Other Studies

A. 1980 Henschler Study

This inhalation study showed no increased incidence of cancer in Syrian hamsters.

B. 1978 MCA Study

An audit of this inhalation study in Charles River rats and $B_6C_3F_1$ mice concluded that it was inadequate to evaluate the presence or absence of a carcinogenic response.[13]

IV. Application of the Guidelines

EPA should describe the animal data as limited instead of sufficient. Many of the effects in animals have not been replicated, are of uncertain biological significance, and show a weak response, even at the high doses used in the bioassays. EPA needs to integrate the mechanism data which have been developed

for trichloroethylene with the bioassay data, in order to develop a plausible hypothesis as to the sensitivity of the mouse relative to the rat and human.

Analysis of the animal bioassay data on trichloroethylene is difficult because of the existence of conflicting results. Two studies have shown an increased incidence of liver tumors in $B_6C_3F_1$ mice. As developed in the following sections, mechanism-of-action studies show that the proximal mouse carcinogen in this case, trichloroacetic acid, does not present a carcinogenic risk to man.

Three inhalation studies show conflicting results in four different strains of mice. An increase in lung tumors was r ported in male Swiss mice and female $B_6C_3F_1$ mice, and in female ICR mice. No increase in lung tumors was reported in either sex of NMRI mice, male $B_6C_3F_1$ mice, female Swiss mice, or male ICR mice. The mechanism by which lung tumors have been produced is not understood, nor is the reason for the conflicting results in various strains. In these circumstances, p nding a determination of the biological significance of the varying mouse lung results, the positive studies should be considered limited, not sufficient, evidence of carcinogenicity in animals.

The draft Addendum emphasizes the Fukuda study as supportive of a finding of sufficient evidence. The Addendum states that the concentration of epichlorohydrin as stabilizer was 0.019%. It does not mention that a number of other putative carcinogenic substances were present as well in the reagent grade of trichloroethylene that was used in this study.

Scientists from the Centers for Disease Control offered the following critique of the Fukuda study in a recent article:[14]

> The incidence of total lung tumors -- that is, adenomas and adenocarcinomas -- was not significantly increased if controls were compared to exposed mice. In addition, the incidence at the higher dose was about the same as that at the lower dose.

In addition, metastases of mammary tumors (in both controls and dosed groups) were observed to spread to the lung. Based on the report, it is not possible to tell whether metastatic tumors were included in the tumor count. However, there was a 12% tumor incidence in the lungs of control mice. At most, there might have been an enhancement of spontaneously occurring lung tumors in dosed animals.

Results in rat bioassays have been negative, with the exception of recent gavage and inhalation studies by Maltoni. Maltoni reported no increase in tumors in female rats. There was an increased incidence of Leydig cell tumors in male rats. Maltoni regarded the Leydig cell tumors as benign, as Leydig cell tumors are microscopically benign in character. Historical data would be useful to determine if there is any evidence that these tumors progress to malignancy. Under the EPA Guidelines, benign tumors are considered limited evidence of carcinogenicity unless they have the potential to progress to an associated malignancy of the same histogenic organ.

There was a slight elevation in renal adenocarcinomas and of leukemias in one exposure group in the male rats in the Maltoni study. These findings are also equivocal, however, and it is by no means clear that they are either meaningful or related to trichloroethylene exposure.

In considering the Maltoni results and their relevance for assessing human risk, it must be emphasized that these studies have never received peer review as is the established practice in the scienctific community in the United States. Rather, the results of the the work were published privately. The other studies in the draft Addendum have generally received scientific scrutiny prior to being reviewed by EPA.

Maltoni breeds his own animals and the particular s nsitivity of these breeds and their historical tumor incidence rates need to be understood to place his most recent results in proper perspective. Moreover, Maltoni follows a procedure differing significantly from the current "state of the art" method for conducting carcinogenicity bioassays. After exposure for 52 weeks, the animals typically are allowed to live until spontaneous death. This may make it difficult to interpret the r levance of late-occurring tumors that would not have been detected at terminal sacrifice taking place at weeks 105 through 107. This may result in counting metastatic tumors rather than tumors which arise de novo in an organ, and thus reporting greater potency than is appropriate.

A workshop was held in Washington, D. C. in December 1985 to discuss Maltoni's recent work. Only a few slides were available for review. The workshop resulted in recommendations for

- o a pathology review of a 10-20% sample of tumors for a quality assurance evaluation, and

- o a detailed statistical analysis of all tumor responses, particularly with mortality correction (this was not done in either the private publication or in the draft Addendum).

A delegation was to have visited Maltoni's laboratory to conduct the pathology review. Since this has never taken place, the questions regarding quality of the data are unresolved.

While the draft Addendum attempts to correct for mortality, more data are needed to adjust for mortality effects. Similarly, a comparison with historical control data is necessary to understand the nature of the Leydig cell tumors and leukemias in these studies relative to Maltoni's rat colony.

In conclusion, the animal bioassay data are either irrelevant to man or conflicting and equivocal. The draft Addendum contains interpretations that support this view in the sections on the selection of animal data sets for quantitative assessment. These interpretations are to some extent inconsistent with the interpretations expressed in the sections on qualitative assessment. The mouse lung results are divided. The unequivocally positive mouse liver results have been shown to be caused by a trichloroethylene metabolite trichloroacetic acid, an effect that would not be seen in humans. The benign Leydig cell tumor increase does not meet the criteria for sufficient evidence. Other assertedly positive responses in male rats reported by Maltoni are too equivocal to be significant. The evidence for trichloroethylene should be considered as limited animal evidence under EPA's guidelines.

Metabolism

The draft Addendum is in error regarding metabolic pathways in man, mouse, and rat. Despite previous advice from the Science Advisory Board to the contrary, the draft Addendum hypothesizes that a reactive epoxide, trichloroethylene oxide, which is thought to be formed as an intermediate during metabolism, is responsible for the mutagenic and carcinogenic ffects of trichloroethylene. Such an epoxide is not an obligatory intermediate in trichloroethylene metabolism.

The major, documented pathway for trichloroethylene metabolism involves the formation first of chloral, followed by conversion to trichloroethanol (reduction) or trichloroacetic acid (oxidation).[15] These products have been demonstrated to occur in the intact animal in the appropriate kinetic sequence.

It is not necessary to postulate "labile, reactive intermediates" that will bind to protein in the trichloroethylene metabolism pathway, since chloral will bind covalently to protein.

The existence of trichloroethylene oxide during in vivo metabolism remains a speculation. If it does exist, it will not occur as a free reactive species. Thus, trichloroethylene oxide has no relevance to the biological effects of trichloroethylene, even if it subsequently is isolated and shown to be an intermediate. The conversion of trichloroethylene oxide to chloral would be catalyzed by hepatic microsomes, and Henschler and coworkers have noted that detoxification of trichloroethylene oxide would occur within the microsomal microenvironment, making the reactive epoxide inaccessible to DNA.[16] The presence of a Lewis acid is necessary for the postulated rearrangement of trichloroethylene oxide to chloral, and microsomal P-450, which would be intimatedly associated on a molecular basis with trichloroethylene oxide as it formed, will serve as a Lewis acid.

Van Dyke (1977) has proposed an alternative, equally plausible hypothesis: direct microsomal oxidation of trichloroethylene to chloral through a chloronium ion transition state.[17] Contrary to the statement in the 1985 HAD, a chloronium ion would have a half-life of less than a microsecond, would not occur separately from the cytochrome P-450 microenvironment (the enzyme pocket), and would not be available to "bind" cell constituents, except cytochrome P-450, covalently.

Speculations in the draft Addendum that carbon dioxide derived from trichloroethylene arises from hydrolysis of free trichloroethylene oxide have been shown to be false. Trichloroacetic acid is the source of carbon dioxide derived from trichloroethylene administration, and trichloroacetic acid formation after trichloroethylene administration is sufficient to account for all carbon dioxide production.

In summary, no evidence exists in favor of a free trichloroethylene oxide intermediate, which might be capable of interaction with DNA. No such intermediate has ever been isolated or demonstrated. The paper cited in the 1985 HAD as evidence of in vitro isolation of trichloroethylene oxide in fact showed the opposite, that trichloroethylene oxide could <u>not</u> be isolated.[18]

While it is reasonable to postulate a trichloroethylene oxide intermediate, based on Daniel's demonstration of intramolecular rearrangement of chlorine[19], such an intermediate would be short-lived and trapped in the cytochrome P-450 binding site. No free trichloroethylene oxide would be available to exert biological effects. As indicated above, an

alternative and equally credible mechanism for conversion of trichloroethylene to chloral through a chloronium ion has been proposed. Chloral and chloral hydrate also can have mutagenic effects albeit with very low potency, so that existence of trichloroethylene oxide is not necessary to explain the known biological effects. Excessive reliance on a hypothetical reactive epoxide leads to excessive speculation, and may be the reason that the sections on pharmacokinetics and mechanism of action incompletely address the scientifically established mechanism of action for the induction of mouse liver tumors (and perhaps for other sites, as well).

Even if EPA decides that trichloroethylene oxide does form the basis of human risk (which is equivalent to the assumption that risk is proportional to total metabolites, if trichloroethylene oxide is an obligatory intermediate in metabolism, as discussed below), EPA's risk assessment does not take into account species differences in epoxide hydrase, which has progressively higher levels in mouse, rat and man.[20/] Epoxide hydrase would detoxify trichloroethylene oxide.

Genotoxicity

The overall interpretation of data and quantitative assessment of risk in the draft Addendum rests strongly on the speculation that carcinogenic effects arise from a postulated intermediate of metabolism in the intact animal, trichloroethylene oxide, that may not exist and cannot exert biological effects. Based on this speculation, total metabolite yield has b en used to estimate risk.

The 1985 HAD states (p. 1-3):

> A conclusion about the mutagenic potential of pure TCI [trichloroethylene] cannot be made. If TCI is mutagenic, the available data suggest that it would be a very weak indirect mutagen.

Trichloroethylene has been tested extensively in many different biological systems, including man. In the 1985 HAD, 19 studies were considered negative, 10 were thought positive and the results of the remaining 17 were not interpretable. Chloral and/or chloral hydrate may be responsible for some of the low potency and marginal mutagenic responses seen in other whole animal systems. The only large increases came in a mouse assay for which there is an alternative interpretation based on peroxisome proliferation. (Peroxisome proliferation leads to increased levels of intracellular hydrogen peroxide, and hydrogen peroxide is mutagenic.) The draft Addendum summarizes new data

since the 1985 HAD, but does not evaluate these studies or integrate this information with the previous evaluation. Six out of seven of the new assays are negative.

Trichloroethylene should not be considered directly genotoxic. While the existing data are difficult to interpret because much testing of trichloroethylene has been done on commercial grades containing stabilizers and impurities, there is a consensus that trichloroethylene is either not mutagenic, or is at most a very weak, indirect mutagen when stabilized. According to Elcombe:[21]

> TRI (trichloroethylene) 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.

Similarly, the 1985 HAD concludes (p. 7-36):

> The available data provide suggestive evidence that commercial-grade TCI (trichloroethylene)is a weakly active, indirect mutagen. Based on this, commercial TCI may have the potential to cause weak or borderline increases above the spontaneous level of mutagenic effects in exposed human tissue. The data on pure TCI do not allow a conclusion to be drawn about its mutagenic potential. However, mutagenic potential cannot be ruled out. If it is mutagenic the available data suggest TCI would be a very weak, indirect mutagen.

This distinction between stabilized and pure trichloroethylene is of considerable importance and also should be emphasized in the Addendum.

EPA has mischaracterized the results of Bergman, who found DNA adducts only in in vitro experiments.[22] The draft Addendum interprets Bergman's in vitro experiments as if they were whole animal adduct studies. This experimental situation differs vastly from one in which animals exposed to trichloroethylene are shown to have DNA adducts. In fact, Bergman did such experiments, found no DNA adducts, and stated the following:

> Aminex A6 chromatography revealed that the entire radioactivity in RNA from liver and kidney and in DNA from kidney, testis, lung, pancreas and spleen was due to metabolic incorporation.
>
> The elution profile of radioactivity in liver DNA gave no direct evidence of the formation of TRI [trichloroethylene]-DNA adducts in vivo. No etheno-derivatives were identified as alkylation products of TRI in vivo, which is consistent with current theories of the metabolic fate of TRI.

The experiments of Stott, Quast, and Watanabe specified a very low upper bound to possible DNA adducts[23], based on the limit of detection, not on actual DNA adducts, as described in the 1985 HAD. This casts serious doubt on the position in the draft Addendum regarding DNA alkylation by trichloroethylene oxide. For that position to be correct, a single covalent molecular interaction between DNA and trichloroethylene oxide would have to be 50,000 times more active than dimethylnitrosamine interacting with DNA or 170,000 times more potent than aflatoxin B-1. In contrast, however, both dimethylnitrosamine and aflatoxin B-1 are highly potent carcinogens and trichloroethylene has very low, if any, potency. This is the reverse of the expectation from the limits of detection of molecular binding.

Further, Keller and Heck recently found that chloral has no potential to bind to DNA or to form DNA-protein crosslinkage, either in vivo or in vitro.[24]

Overall Weight-of-the-Evidence Group

The overall weight of the evidence supports classification of trichloroethylene as a possible human carcinogen in Group C under the EPA Guidelines. The only unequivocally positive results in the bioassays are mouse liver tumors. These constitute "limited" evidence of animal carcinogenicity, at best, in light of the available information showing that trichloroethylene is not genotoxic, that mouse liver tumors result from peroxisome proliferation via the action of a metabolite trichloroacetic acid, and that trichloroacetic acid does not induce peroxisomes in human liver cells. A number of bioassays show mixed results in the mouse lung. The only positive results reported in the rats are either benign, equivocal, or apparently unrelated to trichloroethylene

administration. In addition, the available human epidemiology data do not support classification of trichloroethylene as a probable human carcinogen.

Pharmacokinetics

The presentation of EPA's analysis of pharmacokinetic information interspersed with mechanism-of-action information in the chapter on "oncodynamics" is unnecessary and confusing. This term is neither defined in the text nor in common scientific use. Further, this chapter seems intended to support a quantitative analysis. While integration of the total body of data given the analysis of tumor occurrence is laudable, it is difficult to understand how EPA has developed and selected options for interpretation. Accordingly, the information on pharmacokinetics and mechanism-of-action should each be developed in a separate chapter; then an integration could be put forward.

EPA has had the benefit of reviewing several pharmacokinetic models, such as those by Koizumi and coworkers, Fernandez and coworkers, and Sato and coworkers.[25/] [26/] While two of these reports were available for the 1985 HAD, neither this document nor the draft Addendum lays out the structure of these models. They should be addressed along with a section on quantitative integration of pharmacokinetic information, perhaps in relation to tumor occurrence in different species and known mechanism of action.

In evaluating whether it is biologically plausible that a test chemical shown to produce tumors in experimental rodents would be likely to produce the same response in humans, it is important to integrate both mechanistic considerations and specific known species differences in the pharmacokinetics of the agent. Following exposure to trichloroethylene, blood levels of trichloroacetic acid are 7-fold greater in mice than in rats.[27/] Monster has shown that humans metabolize trichloroethylene at twenty-fold or lower rates than the rat.[28/] In cell culture experiments Elcombe has shown that mouse hepatocytes produce 30-fold more trichloroacetic acid than rat hepatocytes, which in turn produce 3-fold more trichloroacetic acid than human hepatocytes. The rat undergoes biochemical responses to trichloroacetic acid in vitro that are similar to those observed in the mouse, when isolated hepatocytes from the two species are exposed in cell culture experiments. The absence of a carcinogenic effect in the intact rat would appear to be a function of its lower rate of production of trichloroacetic acid. Trichloroacetic acid levels vary between species due to pharmacokinetic differences. This can account for species variation in the induction of tumors in rodents.

Following TCE exposure trichloroacetic acid levels in the human are sufficiently lower than levels observed in the rat, so that tumors would not be expected even if trichloroacetic acid had carcinogenic potential in man.

It is untenable, based on pharmacokinetic information in isolation, to argue that trichloroacetic acid production is a proxy for the total rate of metabolism, and that either a reactive intermediate or a group of metabolites acting in concert causes the biological effects seen in the mouse, but not the rat. These hypotheses are not tenable in the light of evidence from Herren-Freund and coworkers that trichloroacetic acid, when administered directly to $B_6C_3F_1$ mice, causes liver tumors. M chanism-of-action information about the role of trichloroacetic acid in the proliferation of peroxisomes in rodent liver as a marker for (or essential step in) carcinogenesis also supports the essential role of trichloroacetic acid.

The draft Addendum becomes sufficiently caught up in a theory of total metabolites that it misinterprets two experiments and cites the misinterpretations as evidence against an essential role for trichloroacetic acid. The quantitative doses (and tumor yields) used in the experiment by Herren-Freund and coworkers do not contradict the essential role for trichloroacetic acid. The draft Addendum seems to miss the point that concentration of trichloroacetic acid formed in situ in liver cells after external administration of trichloroethylene cannot be directly equated with the externally applied dose of trichloroacetic acid. Surely, this is the point of pharmacokinetic analysis.

A similar error is made in interpreting the data of Goldsworthy and Popp.[29/] The draft Addendum states (at 4-5) that Goldsworthy and Popp found that trichloroethylene is more potent than trichloroacetic acid as a peroxisome proliferator. This is not accurate. Goldsworthy and Popp reported that 1000 mg/kg of trichloroethylene increases peroxisome levels 625% in male $B_6C_3F_1$ mice and 360% in female mice, whereas 500 mg/kg of trichloroacetic acid increased male peroxisome levels by 280% and female by 305%. Beyond the lack of parallel comparison between dose and/or biological effect, the draft Addendum erroneously equates in situ concentrations of trichloroacetic acid in liver cells after external administration of trichloroacetic acid.

Mechanism-Of-Action

The most significant finding to come out of the many long-term animal carcinogenicity studies of trichloroethylene is that it produces liver cancer in mice, but not in rats or hamsters. Mechanism studies, described below, have shown that

these tumors arise by a mechanism specific to rodents. Thus, humans are unlikely to be at risk from trichloroethylene-induced liver cancer.

Hepatocarcinogenic effects similar to those seen in the mouse have not been observed in the rat. Because the rat and the mouse are much more closely related to each other than either is to the human, the lack of replication of the mouse findings in the rat indicates that automatic extrapolation of the $B_6C_3F_1$ liver tumor response to man is not appropriate. The difference between rodent species apparently is explained by quantitative differences in the production of trichloroacetic acid, which can cause liver tumors in $B_6C_3F_1$ mice when directly administered.

Mouse liver tumor formation is associated with the proliferation of peroxisomes. For trichloroethylene this proliferation is due to a metabolite, trichloroacetic acid, which is the proximal carcinogen. Trichloroacetic acid alone can cause proliferation of peroxisomes in mouse liver cells in culture or hepatic tumors in the intact mouse. The level of peroxisome proliferation in the mouse (and the rat) corresponds closely with the level of trichloroacetic acid production. Herren-Freund and coworkers have shown that trichloroacetic acid alone acts to induce mouse liver tumors in the $B_6C_3F_1$ mouse. Trichloroacetic acid does not cause peroxisome proliferation in human liver cells in culture and there is no evidence of derangement of lipid metabolism in humans exposed to trichloroethylene, as there is in mice whose peroxisomes are proliferated by a variety of agents.

Elcombe has shown that peroxisome proliferation does not occur in human hepatocytes following in vitro exposure to trichloroacetic acid, whereas it does occur in rodent hepatocytes. Thus, humans are unlikely to show a carcinogenic response to trichloroethylene, because they fail to demonstrate biochemically the critical response, the proliferation of peroxisomes.

Trichloroacetic acid is one of a large number of substances known to cause peroxisome proliferation and tumors in the liver of the $B_6C_3F_1$ mouse. These substances do not appear to induce peroxisome proliferation in humans and some of them have been used in human medicine over substantial periods of time with no evidence of human carcinogenicity.

It is too soon after the recent reports of rat tumors associated with trichloroethylene administration for the scientific community to have developed working hypotheses regarding mechanism of action. The speculation in the draft

Addendum that the yield of these tumors is related to the total yield of metabolites is inconsistent with the failure to observe these tumors in mice.

Speculations for rat testes and mouse lung that these tumors arise from a specific trichloroethylene metabolite are attractive working hypotheses for experimental purposes, which have at least as much credence as the speculations on which EPA's approach is based. In the case of mouse liver, EPA's speculation directly contradicts the experimental evidence from several laboratories that trichloroacetic acid, and only trichloroacetic acid, is the proximal rodent carcinogen.

To offset this contradiction, the draft Addendum puts forward the idea that quantitative assessment based on the total yield of metabolites is preferred because different metabolites could act at different stages of carcinogenic progression. This is unfounded. A single substance which is not metabolized in any way can still act at multiple stages of carcinogenic progress, and the multistage model of carcinogenesis does not require acceleration of more than one stage.

Therefore, EPA should calculate tumor yields in relation to body burden (or even better, organ burden) of each individual metabolite. EPA should base quantitative assessment of carcinogenic risk for humans on physiologic levels of specific metabolites after external exposure to trichloroethylene, although the draft Addendum might portray risks based on total metabolite yield for purposes of discussion.

Critique of "Linearized Multistage" Model and Suggested Improvements

In the draft Addendum EPA should use body weight per unit time, not body surface area, as the basis for dose adjustment, if the Agency persists in using the so called "linearized multistage" or "Crump" model for risk estimation purposes. EPA also should replace this model with a procedure having more biological motivation, such as a Moolgavkar-Knudson model.

EPA's current guidelines for carcinogen risk assessment call for the development of a biologically motivated model to estimate quantitative risk, even when this calculation is carried out as a "what-if" exercise. EPA has not made such an effort in the case of trichloroethylene, although an appealing alternative to the model in the draft Addendum is within the reach of EPA's scientists.

EPA's current methodology for quantitative risk assessment is dominated by two policy assumptions (1) that carcinogens do not have practical thresholds, and (2) that carcinogenic risk is a linear function of dose even at very low doses.[30] Given these policy choices, EPA has chosen a mathematical model that performs well in estimating a maximum linear slope consistent with bioassay data. The model, sometimes referred to as the "linearized multistage" or "Crump" model, takes incidence data obtained at all doses into account, whereas "straight-line" or "single-hit" (Poisson) models have difficulty accepting more than one data point.[31] The Crump model operates with the data from a bioassay as follows:

- o A version of the multistage model is developed that mathematically resembles a true multistage model, with the number of stages constrained by the number of non-zero doses used in the bioassay.

- o A maximum likelihood fit of this specific model to the bioassay data is developed with the exponential values for each stage constrained to give only positive risks.

- o All exponential coefficients higher than the single-hit (linear) component are held fixed and the magnitude of the single-hit exponent is enlarged in the direction of increasing risk to obtain a maximum value compatible with the data in a 95% confidence limit sense. The value of the linear exponent the slope is used for risk estimation purposes.

However, the Crump model is not without difficulties. It is changed sufficiently from the original model of Armitage and Doll that is no longer retains a biological rationale.[32] The stages in the Crump model do not relate to discrete modifications of a cell line in the pathway to an observable tumor, and the number of stages is not related to the number of stages in the carcinogenic process. The exponents do not relate to the times between these discrete cell variants, and the overall set of exponential coefficients do not relate to the time to tumor. The constraint on non-negative exponents means that models in which a substance lengthens the time of one stage are forbidden. However, this biological effect has been observed experimentally. In short, a Crump model for a substance is not derived from an underlying biological theory of carcinogenesis or from knowledge of relevant biological effects of the substance in question. Instead, this model is a curve-fitting mechanism.

The curve fitting exercise is not without its costs. Point values are produced which tend to be insensitive to changes in the shape of the dose-response curve. The confidence limit-driven slope is more sensitive to the number of animals the investigator may choose for the bioassay than to the tumorigenic response. The number of animals used is an irrelevant variable for a model of the carcinogenic effect of a substance on which regulations will be based. A Crump model accepts pharmacokinetic or time-to-tumor data only with difficulty. Mathematical curve-fitting ignores metabolic features such as alternative pathways, competing pathways, saturation, and so forth. Information on age-specific cancer incidence, background rates, including cell-turnover or cell population kinetics, and lack of mutagenicity cannot be used at all unless the model maker arbitrarily alters the parameters.

Instead of a Crump model for trichloroethylene, such as that described in the draft Addendum, EPA could develop a Moolgavkar-Knudson model.[33]

A Moolgavkar-Knudson model describes cancer induction as a filtered Poisson process with deterministic and stochastic elements that account for the dynamics of a cell population that is intermediate between two stages, transition from normal cells and transition to malignant cells. Biologically, these two transitions are characterized as rare and irreversible in practice. Use of a Moolgavkar-Knudson model for trichloroethylene offers many advantages for EPA's scientists.[34] Moolgavkar-Knudson models do have a biological rationale and suggest research directions to improvement of the risk estimates. A Moolgavkar-Knudson model can be constructed that fits the data and that does not contradict EPA's major policy assumptions of no practical threshold and low dose linearity. For example, a Moolgavkar-Knudson model can account for an unusual age-specific incidence of tumors that exhibits a high incidence during a specific age period and then declines, such as childhood or hormonally dependent cancers. Further, EPA can model some of the pharmacodynamic factors involved in dose adjustment between species, by relating human background rates and age-specific incidence to those of the rodents used as bioassay subjects. Pharmacokinetic data, suggested mechanisms (e.g. promotion versus initiation), and time to tumor are scientifically usable to modify the parameters of the model.

Many of the advantages of the Moolgavkar-Knudson model over the Crump model are directly relevant to the specific properties of trichloroethylene, such as (1) shape of the dose-response curve, (2) low incidence at maximal response doses, (3) high background tumor rates in the mouse strains used as bioassay subjects, (4) strong suggestions of action by a promotional,

non-genotoxic mechanism, (5) pharmacokinetics and mechanism-of-action data that have strong nonlinearities, (6) lack of genotoxicity and (7) species differences.

Scientists from HSIA member companies do not suggest that the Moolgavkar-Knudson model is the best description of carcinogenic risk from trichloroethylene (or indeed, any other chemical). In many cases, and trichloroethylene is one, metabolic differences between species strongly suggest that despite rodent carcinogenicity there may be no human hazard. The OSTP guidelines advise that such pharmacokinetic data should, when available, be incorporated into a risk assessment. In such cases, EPA would need to abandon the two policy assumptions of no threshold and low-dose linearity. Recognizing that such a state-of-the-art scientific approach may need time to gain general acceptance, as an interim measure EPA can use a Moolgavkar-Knudson model as a biologically more appropriate description that does not require the Agency to abandon the two traditional policy assumptions.

Should EPA persist with the use of the Crump model to estimate the "what-if" risks for trichloroethylene, a better choice of dose adjustment factor is available than body surface area, as described in the draft Addendum and discussed at the Science Advisory Board meetings on August 11-14. Optimally EPA will use a physiological pharmacokinetic model for purposes of dose adjustment. Failing this, in the final Health Assessment Document and in the draft Addendum, dose conversion should be carried out by body weight, which provides a better basis for dose adjustment between species. EPA has cited some values that are coincidentally similar to justify the use of body surface area means of a pharmacokinetic model. Those calculations have no real bearing on the choice of an appropriate dose adjustment factor and are contrived. Two kinds of data are available to EPA that have crucial impact on the best choice of a "default" value, as defined by the National Academy of Sciences Committee on Institutional Means for Risk Assessment.[35]

First, EPA recently co-sponsored a comparison of carcinogenic potency of various substances in humans and rodents. While the correlation between human and rodent potencies obtained with body weight as the dose adjustment basis was similar to that with body surface area (0.70 versus 0.71), for prediction of the potency value, body weight proved far superior to body surface area.[36] Second, two groups of investigators have tested the ability of the mouse to predict the rat and vice-versa. Both Wilson, Crouch and coworkers[37] and Gaylor and Chen[38] found that body weight proved superior to body surface area in predicting potency. In the calculations in the draft Addendum, EPA has introduced a number of modifications

to the usual procedure to develop a Crump model, obtained values that crudely agree on a body surface area basis, and then used this agreement as a justification for the use of body surface area. EPA instead should carry out calculations without major modifications using body weight for dose adjustment purposes and then attempt to discover the reasons why there are discrepancies between potency estimates, if any occur.

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ICI Chemicals and Polymers Group
Occupational Health Department
P.O. Box 13 The Heath RUNCORN Cheshire WA7 4QF
Telephone Runcorn (0928) 51 3953 Direct Line
Runcorn (0928) 514444 Switchboard
Telex 629655 ICIMOH G

C&P Chemicals and Polymers Group | Occupational Health Department

HEALTH AND SCIENCE
TO: COMMITTEE
FROM: TOM CORTINA

NEW FILE

FILING INSTRUCTIONS:
H/H/T
HSIA
Health + Science Comm
Fiechter - EPA/HA

Project Officer for Trichloroethylene (C)
Office of Health and Environmental Assessment (RD-689)
Room 3812
US Environmental Protection Agency
Waterside Mall
401 M Street SW
Washington DC 20460
U S A

Your ref

Our ref
DGF/CES

Dear Sir

Submission on draft report EPA/600/8 - 82/006FA
"Addendum to the Health Assessment Document for
Updated carcinogenicity assessment for trichlor

I respectfully submit comments on the above dra
consideration by the EPA. These comments are ma
Chemicals and Polymers Group, the major UK producers of trichloro-
ethylene. I also comment on behalf of my colleagues at the ICI Central Toxicology Laboratory, in particular Dr Clifford R Elcombe, who has conducted studies which have addressed the relevance to human risk assessment of the observed hepatocarcinogenic response of the mouse to trichloroethylene.

Our overall impression of the document is that it has not taken proper account of all of the information pertinent to the hazard assessment of trichloroethylene for man. Furthermore, the document misrepresents the literature in certain instances. The review also implies, in several instances, that trichloroethylene should be viewed in the same light as vinyl chloride, a known human carcinogen whose mechanism of action has been unequivocally shown to be related to a classical genotoxic event. The available information clearly demonstrates that there is no similarity between the mechanism of the carcinogenic response in the rodent to vinyl chloride and to trichloroethylene. On the contrary, the available evidence on trichloroethylene demonstrates clearly that its carcinogenic effect in rodents is mediated by a non-genotoxic mechanism. As such, it is misleading to consider it in the same light as vinyl chloride.

The purpose of this submission is to register with the EPA our view of the mechanism of the rodent hepatocarcinogenic response of trichloroethylene and also to detail some inaccuracies in the draft report.

SL 037913

ICI Chemicals and Polymers Group
Occupational Health Department
P.O. Box 13 The Heath RUNCORN Cheshire WA7 4QF
Telephone Runcorn (0928) 51 3953 Direct Line
Runcorn (0928) 514444 Switchboard
Telex 629655 ICIMOH G

C&P Chemicals and Polymers Group | Occupational Health Department

HEALTH AND SCIENCE
TO: COMMITTEE
FROM: TOM CORTINA
DATE: 9/25/87

Project Officer for Trichloroethylene (C)
Office of Health and Environmental Assessment (RD-689)
Room 3812
US Environmental Protection Agency
Waterside Mall
401 M Street SW
Washington DC 20460
U S A

Your ref | Our ref DGF/CES | SEP 28 1987 | Date 4 Sep 87

Dear Sir

Submission on draft report EPA/600/8 - 82/006FA
"Addendum to the Health Assessment Document for trichloroethylene.
Updated carcinogenicity assessment for trichloroethylene

I respectfully submit comments on the above draft document for consideration by the EPA. These comments are made on behalf of ICI Chemicals and Polymers Group, the major UK producers of trichloroethylene. I also comment on behalf of my colleagues at the ICI Central Toxicology Laboratory, in particular Dr Clifford R Elcombe, who has conducted studies which have addressed the relevance to human risk assessment of the observed hepatocarcinogenic response of the mouse to trichloroethylene.

Our overall impression of the document is that it has not taken proper account of all of the information pertinent to the hazard assessment of trichloroethylene for man. Furthermore, the document misrepresents the literature in certain instances. The review also implies, in several instances, that trichloroethylene should be viewed in the same light as vinyl chloride, a known human carcinogen whose mechanism of action has been unequivocally shown to be related to a classical genotoxic event. The available information clearly demonstrates that there is no similarity between the mechanism of the carcinogenic response in the rodent to vinyl chloride and to trichloroethylene. On the contrary, the available evidence on trichloroethylene demonstrates clearly that its carcinogenic effect in rodents is mediated by a non-genotoxic mechanism. As such, it is misleading to consider it in the same light as vinyl chloride.

The purpose of this submission is to register with the EPA our view of the mechanism of the rodent hepatocarcinogenic response of trichloroethylene and also to detail some inaccuracies in the draft report.

SL 037914

Studies at the ICI Central Toxicology Laboratory have examined the mechanism of action of trichloroethylene as a hepatocarcinogen in the mouse and have addressed the relevance of this observation for human risk assessment. These studies have generated both pharmacokinetic and qualitative data which dispute the relevance of the response for man. These data, coupled with a growing body of supporting evidence in the literature, leads us to conclude that trichloroethylene presents no significant hepatocarcinogenic hazard to man. The evidence supporting this hypothesis is presented in the enclosed publication by Dr Elcombe "Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: a biochemical human hazard assessment". These data have been presented to the US Scientific Advisory Board by Dr Elcombe. We concluded that the Board members acknowledged the pertinence of our arguments.

Our remaining comments are addressed to specific points of concern in the report.

Page 2-9. It is stated that trichloroethylene is metabolised to an epoxide, an intermediate metabolite, and that the majority of the metabolism goes through this route. Whilst this may be true in principle, the epoxide intermediate is only transient and is of no biological significance in the mechanism of the carcinogenesis of trichloroethylene.

It is also stated that humans appear to metabolise trichloroethylene extensively and more so than animals. This statement is incorrect. The evidence is that humans metabolise trichloroethylene less extensively than animals, in particular those species in which tumours have been demonstrated e.g. the mouse. In this respect one should consider the well documented species differences in saturation of trichloroethylene metabolism.

Page 2-10. Chloroform is listed amongst the reactive intermediates found during the metabolism of trichloroethylene. Chloroform is not a biologically significant metabolite of trichloroethylene. In addition, this fact has not been referenced by the authors.

Page 2-12. The reference to Green and Prout (1985) has been misrepresented. In the study, only the mice were dosed for 180 days with trichloroethylene at 1000 mg/kg/day. However, in the same paper an experiment is described in which both rats and mice received a single dose of trichloroethylene in which its metabolism was compared.

Page 2-13. The second paragraph on this page implicates the role of the reactive metabolites of trichloroethylene in its toxicity. Elcombe's work has demonstrated unequivocally that the major metabolites of trichloroethylene are trichloroacetic acid and trichloroethanol. Neither of these metabolites are considered to be reactive species. However, the work of Elcombe and others clearly implicates a role for trichloroacetic acid in the hepatotoxicity and the hepatocarcinogenicity of trichloroethylene. The authors' intention in disregarding these important observations is unclear.

Page 2-14. The second paragraph on this page describes the interaction between drugs and other xenobiotic agents and the metabolism of trichloroethylene. This phenomenon is of no significance in the carcinogenic hazard assessment of trichloroethylene for man.

Page 3-12. The report contains an extensive review of the carcinogenicity studies conducted by Maltoni. He reported an increased incidence of Leydig cell tumours in male rats exposed to trichloroethylene by inhalation. It should be noted that this tumour type has not been observed in other carcinogenicity bioassays of trichloroethylene in the rat. Furthermore, the tumours (with a single exception) were benign in character. These two facts lead us to the conclusion that this isolated finding is of little significance in the carcinogenic hazard assessment an trichloroethylene.

Similarly, Maltoni reported a slight increase in the incidence of immunoblastic lymphosarcomas in both male and female rats exposed to trichloroethylene by inhalation. It is our opinion that this observation is an incidental finding and was not related to exposure to trichloroethylene.

Page 3-40. In the third paragraph, it is stated that the production of trichloroacetic acid and dichloroacetic acid, both shown to be liver carcinogens by the work of Herren-Freund et al, should be considered as prima facie evidence of human risk. The work of Elcombe shows unequivocally that this statement is invalid. The fact that the mouse, in particular, metabolises trichloroethylene to trichloroacetic acid is the very reason for that species, and not others, to show a hepatocarcinogenic response. The reason for the species differences, as explained by Elcombe, is the relative sensitivity to the hepatotoxic effects of trichloroacetic acid (in particular, peroxisome proliferation) with the mouse being the most sensitive species studied to date and with humans showing little, if any, response.

Pages 3-40 and 3-42. The authors make two references to the work of Bergman who had indicated that the DNA adduct, 1, N^6-ethano-adenine, had been found in the liver of mice exposed to trichloroethylene. What the authors of the report have failed to highlight is that, in the same paper, Bergman concluded that "the elution profile of radioactivity in liver DNA gave no direct evidence of the formation of TRI-DNA adducts in vivo. No etheno-derivatives were identified as alkylation products of TRI in vivo", which is consistent with current theories of the metabolic fate of TRI. It is also pointed out that negative DNA binding studies on trichloroethylene have also been published by various authors e.g. Parchman & Magee, 1982, J. Toxicol. Environ. Health, 9, 797-813; Stott et al. 1982, Toxicol. Appl. Pharmacol: 62, 137-151.

Page 4-4. The authors have quoted the wrong reference to Reddy's work in which he reported an increase in peroxisomes among primates after the administration of hypolipidemic agents. The correct reference is Reddy et al., 1984, Am. J. Pathol., 114, 171-183.

However, we would point ut that other workers have shown a lack of response in primates (e.g. Elcombe & Mitchell, 1986, Environ. Health Persp. Vol 70, 211-219; ELcombe 1985, enclosed). Furthermore, Reddy's own work does indicate that there is a marked difference in sensitivity between th species in their response to peroxisome proliferating agents, with primates being the least sensitive. The important conclusion from these studies is not necessarily that primates (humans) do not respond to peroxisome proliferators, but that if they do respond, the sensitivity of the response is markedly reduced when compared to the mouse.

In the last paragraph on this page, the authors equate the increased cellular proliferation observed following the administration of trichloroethylene with the increased cell turnover observed with alkylating agents. It is pointed out that the mechanism of the two effects is entirely different. The cellular response to alkylating agents is a regenerative hyperplasia in response to cytotoxicity. However, the response to trichloroethylene is proliferative in nature and is not associated with cytotoxicity.

Page 4-5. The second paragraph completely misquotes the work of Goldsworthy and Popp. This matter was discussed extensively at the recent SAB meeting and has therefore been drawn to the attention of EPA staff.

Page 4-6. The authors have failed to identify the references supporting the statements concerning the testicular toxicity of trichloroethylene (second paragraph). As stated earlier, we believe that the Maltoni finding of benign testicular tumours is of little relevance to the issue being addressed.

Page 6-7. It is pointed out that the carcinogenicity studies published by Henschler, which showed no tumour response to trichloroethylene, are not included in the overall review of the available data. These studies are referred to earlier in the report and must be taken into account in the overall assessment of the carcinogenicity of trichloroethylene in laboratory animals.

Our comments on this report are concluded. It is our opinion that the report requires substantial redrafting before it can be accepted as a fair review of the available literature on the carcinogenic potential of trichloroethylene for man. In that drafting, due account must be taken of recently emerging hypotheses concerning the mechanism of the hepatocarcinogenic effect of trichloroethylene in the mouse and its relevance to man.

I trust that our comments are of value to you when reviewing this report.

Yours faithfully

SL 037917

D G Farrar

ICI Chemicals and Polymers Group
Occupational Health Department
P.O. Box 13 The Heath RUNCORN Cheshire WA7 4QF
Telephone Runcorn (0928) 51 Direct Line
Runcorn (0928) 514444 Switchboard
Telex 629655 ICIMOH G

C&P Chemicals and Polymers Group | Occupational Health Department

Project Officer for Chlorinated Solvents [E]
Office of Health and Environmental Assessment [RD-689]
Room 3817
US Environmental Protection Agency
Waterside Mall
401 M Street SW
Washington DC 20460
USA

Your ref	Our ref	Date
	DGF/CES	4 Sept 87

Dear Sir

Submission on EPA draft documents EPA/600/8-87/029A and

EPA/600/8-87/030A concerning dichloromethane (methylene chloride)

I respectfully submit comments on the above draft documents for consideration by the EPA. These comments are made on behalf of ICI Chemicals and Polymers Group, the major UK producers of methylene chloride. I also comment on behalf of my colleagues at the ICI Central Toxicology Laboratory, in particular Dr Trevor Green, who has conducted a major toxicology research programme of relevance to the issues addressed in the documents. This research programme has been conducted under the sponsorship of CEFIC on behalf of the European producers of methylene chloride. EPA staff are aware of the content and results to date emerging from this programme.

1) EPA/600/8-87/029A HRAC
'Technical analysis of new methods and data regarding dichloromethane hazard assessments'.

This report reflects the opinion of the HRAC of the basic scientific data on methylene chloride that impinges on its carcinogenic risk assessment for man. Our own review and interpretation of this basic data is contained in ECETOC Technical Report No 26 "The assessment of carcinogenic hazard of human beings exposed to methylene chloride", which I enclose. This report was written by Dr Green and Dr I F H Purchase, Director of the ICI Central Toxicology Laboratory, and subsequently reviewed and agreed by an ECETOC Task Force, the membership of which can be found on page 59 of the report.

Chapter 5 of the HRAC report deals specifically with a review of mutagenicity studies on methylene chloride performed by CEFIC. Comments on Sections 5.1.1 and 5.1.2 of this chapter, which reviewed the in-vitro and in-vivo UDS assays performed by CEFIC, are contained in Appendix I to this letter. These comments have been received by EPA staff in the past and I include them with this letter for your convenience.

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We note that HRAC have also made extensive comments on the DNA-binding and S-phase studies performed by CEFIC. Our response to these comments will be submitted to EPA in due course.

Further comments relate to HRAC's criticism of the mouse micronucleus test performed by CEFIC (pg. 75 - CEFIC, 1986d). This study was conducted using the best available assay system according to OECD test guidelines. The assay is accepted on an international basis to be a valid part of a tier-testing approach to detect genotoxicity. The lack of an effect of methylene chloride in this adequately performed assay is an important part of evidence that leads to the conclusion that the compound does not possess genotoxic activity in in vivo mammalian assay systems.

2) EPA/600/8-87/030A
'Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride): pharmacokinetics, mechanism of action and epidemiology'.

Our general impression of this document is that it has been well prepared and that it addresses all relevant aspects of the carcinogenic risk assessment of methylene chloride for man. In particular, we are pleased to see that EPA acknowledge the value of the use of pharmacokinetic data on methylene chloride in a physiological risk assessment model of the type described by Anderson and Reitz. We concur with the EPA that the preferred method for estimating the internal dose of methylene chloride in man should be based on allometric considerations (ie. Method 1) thus arriving at a more relevant estimation by extrapolation between species. Furthermore, we agree with EPA that the generation of improved pharmacokinetic data on methylene chloride will further validate the value of such a model in the carcinogenic risk assessment on methylene chloride.

On several occasions, the report acknowledges that further work is being conducted under the CEFIC research programme on methylene chloride. The update of the most recent results of these studies is enclosed (ECETOC Statement No 4 - June 1987: current results from European Chemical Industry - sponsored research into species differences in the toxicology of methylene chloride). Final reports of these studies will be available in October 1987.

Our detailed comments on the draft EPA report are as follows:

a) Significance of rat mammary tumours for man (page 5).

It is stated that "the NTP carcinogenesis bioassay clearly demonstrates that DCM is oncogenic in two species of laboratory animals, rats and mice .." Whilst accepting the conclusion that methylene chloride is carcinogenic in the mouse, the conclusion for the rat is dependent solely on the occurrence of benign mammary tumours at a marginally significant rate. The relevance of this observation to the overall assessment of the carcinogenicity of methylene chloride in laboratory animals, and thus its potential carcinogenicity for man, is challenged. The arguments leading to the conclusion that the occurrence of these hormone-dependent mammary tumours in the rat are of no relevance to low dose exposure in humans are presented in ECETOC Technical Report No 26 (pages 20-21).

b) Blood/fat partition coefficients (page 16).

It is stated that the final results of the CEFIC work on blood/fat partition coefficients are expected to be available in th summer of 1987. These data are being generated and will be used by CEFIC as input to the physiological model along with the improved pharmacokinetic data and other physiological parameters (eg.

c) Assessment of GST activity in human liver in vitro (page 21).

Further analyses of GST activity in human liver samples have now been performed by CEFIC, bringing the group size to 10. The estimate of the rate of metabolism of methylene chloride by the GST pathway in human liver cells, measured with Cl^{16} methylene chloride, is now 0.04-0.96 nmol/min/mg protein at a substrate concentration of 35 mM (cf ECETOC Statement No 4).

d) Ongoing CEFIC studies.

Several references are made in the report to CEFIC studies which are ongoing. The current status of these studies is reported in ECETOC Statement No. 4. The final reports of these studies will be available in October 1987 and they will be submitted to EPA.

In brief, the relative rates of metabolism of methylene chloride by the GST pathway in mouse, rat, hamster and human liver have now been established by an improved methodology using radioisotopes of methylene chloride (page 24-25).

New experimental values for blood/fat partition coefficient, time volumes, breathing rates and metabolic parameters are being developed (page 28). In particular, stable isotope studies have shown unequivocally that CO_2 is derived from methylene chloride when metabolised by both the GST and the P_{450} pathways, with over 70% of the CO_2 being derived from the latter pathway at low dose levels. This observation has been taken into account in the derivation of in vivo pharmacokinetic rate constants (Km and Vmax) in relevant strains of rats and mice over a range of five dose levels (100-4000 ppm).

The ability of the physiological model to predict the behaviour of methylene chloride in man will be validated by an interspecies comparison of in vitro and in vivo pharmacokinetic constants. The model has been used successfully to predict the pharmacokinetic behaviour of methylene chloride in both the mouse and the rat, using data from the alternate species. Thus, we are confident that the model can also be used to predict accurately the pharmacokinetic behaviour of methylene chloride in the hamster and in man on the basis of metabolic constants determined experimentally in vitro using relevant tissues.

With respect to the physiological model, we are confident that the input of the new experimental data will lead to improvements in the current model, resulting in a better risk assessment. However, we have residual concerns about the current treatment of the lung as a single compartment in the model, but will have addressed this matter by the year end.

Further reference to ongoing CEFIC work is made on pages 61/62 concerning the assessment of GST activity in human liver and human lung tissue. The current status of our work in human liver is referred to in ECETOC Statement No 4. The final report of this work will be available in October 1987.

e) The role of the Clara cell in the mouse lung tumour response.

Further work has been conducted to study the role of the Clara cell in the g nesis of lung tumours in the mouse. The studies are referred to in ECETOC Statement No 4 and will be fully reported in October 1987.

It is known that after one day's exposure to methylene chloride, a selective cytotoxic effect in the Clara cell occurs. In addition, all membrane-bound enzyme activity in damaged Clara cells appears to be lost. However, following a 10-day exposure, the lesion disappears and a selective recovery of P_{450} enzymes can be demonstrated. Recent studies have shown that the methylene chloride P_{450} isoenzym does not recover, however. The activity of the cytosolic GST is not affected during this time course. Thus it appears that the 'protective' effect of the P_{450} pathway is lost to the Clara cell during this time course, leaving the GST route as the only route of metabolism. These observations are taken as further evidence that the Clara cell is the cell of origin of the lung tumours in the mouse.

f) Evidence for genotoxicity.

We do not agree with EPA's conclusion that methylene chloride may be a weak mutagen in mammalian systems and that this mechanism should be considered as a possible cause of the carcinogenic response.

We accept that methylene chloride causes mutations in bacterial assay systems. However, we conclude that this response is associated with the metabolism of methylene chloride to an active species by bacterial enzymes. The relevance of this observation to the overall genotoxicity of methylene chloride is discussed in ECETOC Technical Report No 26 (pgs 7-17). The EPA have concluded that this genotoxic activity has not been detected in the <u>in vivo</u> assay systems employed due to their insensitivity. Our own interpretation of the totality of the data is that despite numerous attempts using a range of validated <u>in vivo</u> assay systems in a number of institutions, using relevant dose levels and relevant strains of rats and mice, it is not possible to demonstrate genotoxic activity with methylene chloride in <u>in vivo</u> mammalian systems. Whilst we accept that it is almost impossible to prove a lack of such an effect beyond doubt, we conclude that the weight of evidence suggests that methylene chloride is devoid of genotoxic activity in <u>in vivo</u> mammalian assay systems and is thus unlikely to be mutagenic in man.

We accept that, as a consequence, we have no explanation for the mechanism of the carcinogenic effect of methylene chloride in the mouse, except to say that there is no evidence for a mutagenic effect of the parent compound or its metabolites. Thus, we are content to leave this question open, rather than to conclude, in the absence of any alternative explanation, that the chemical possesses weak genotoxic activity which is yet to be detected.

g) Relative responsiveness of mice and humans to the calculated internal dose (pages 55-58).

The EPA have concluded that a 12.7 factor must be applied to the estimated internal dose of methylene chloride in mice to take account of differences in responsiveness between mice and humans. The validity of this approach is open to debate. Alternative factors, such as the one applied by Anderson and Reitz, may well be equally justified.

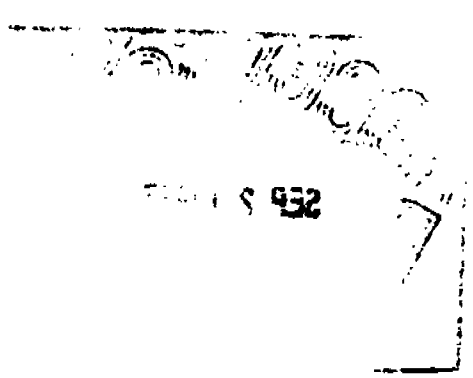
h) Impact of epidemiology.

The EPA view of the impact of the epidemiological information on methylene chloride is, for the most part, not unreasonable. However, we would point out that the HRAC analysis of the incidence of pancreatic tumours in the Eastman-Kodak study was based on the assumption that there was significance to the presumed dose-response trend in the data. Thus, it became possible to calculate an increased SMR for pancreatic tumours in the exposed population. The use of trend analysis statistics in this way is questioned.

I trust that you will take our comments into account in your further consideration of the carcinogenic risk assessment on methylene chloride. In particular, I ask you to remain aware of the continuing CEFIC work programme on methylene chloride and request that you defer any subsequent consideration on the chemical until the results of those studies are finally reported.

Yours faithfully

D G Farrar
Regional Toxicologist



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